UNIVERSITY OF BUCHAREST

FACULTY OF CHEMISTRY

DOCTORAL SCHOOL OF CHEMISTRY

ABSTRACT OF PhD THESIS

COMPLEXES OF SOME 3d TRANSITION METALS WITH AZOLE TYPE LIGANDS, AS MATERIALS WITH BIOLOGICAL ACTIVITY

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INTRODUCTION

Studies related to heterocyclic compounds of azole type ocupy an important place in medicinal chemistry, these type of compounds being studied due to their multiple pharmacological activities confirmed by numerous literature data. These species are used in coordination chemistry since 19th century, once the desire of quantification of pharmacological effects and diminuation of secondary effects. The coordination versatility of the carboxylate group justifies the choice of carboxylic acids beside azole type ligands, these first mentioned making possible in a great extend the existance of a large number of complexes of all types: mono- and polynuclear, with polymeric structures and supramolecular interactions.

PhD thesis entitled "**Complexes of some 3d transition metals with azole type ligands, as materials with biological activity**" approaches the synthesis and characterization of some new complexes which contain as ligands the acrylic acid and different simple azole type ligands.

The PhD thesis is structured in two main chapters, the first one is an extensive refenced study regarding synthesis, characterisation and structural analysis of complexes with azole type ligands and carboxylic acids. The second chapter presents a detailed desciption of the original results, synthesis and analysis methods of the obtained compounds.

It was synthesized and characterised a number of **49** complexes which contain as metallic ions Co(II), Ni(II), Cu(II), acrylate ion and different ligands of azole type as ligands: pyrazole/pyrazole derivatives, imidazolee/imidazolee derivatives, benzimidazole/benzimidazole derivatives.

The new compounds were characterised by elemental analysis, IR and UV-Vis spectroscopy. The redox behaviour was investigated through cyclic voltammetry, while the thermal decomposition studies elucidated complexes composition as well as the presence, number and nature of water molecules.

The applied methodes for single crystals obtaining to establish without doubt the complexes crystalline structure of gave results, thus was obtained the crystalline structure of six complexes through X-ray diffraction analysis on single crystals.

Another objective of this PhD thesis was the highlighting of the antimicrobial activity of the synthesized complexes. With respect to this, the coordination compounds were subjected to a qualitative screening through difuzimetric method followed by quantitative analysis through microdilution method against a series of Gram-positive and Gram-negative bacterials strains and fungus. The research carried out for the fullfillment of this objective concluded that most of the complexes presented antimicrobial activity with small values of the minimal inhibitory concentrations, meaning that they can be used as antimicrobial agents.

It was analysed the compounds influence on the microbial biofilms development on inert substrate, observing that these compounds have an inhibitory effect on inert substrate adherence of the studied bacterial strains, in a great measure covering a large concentrations domain.

Cytotoxicity studies confirmed the possible usage of the biologically active compounds, otherwise due to their high cytotoxicity the possible usage of these complexes as anticancer agents.

Studies regarding the antiinflammatory activity evidenced the possible antiinflammatory effects of copper(II) complexes.

Systems used in complexes syntheses:

Complexes were obtained through "one pot" method:

- M(II) : acrylic acid : pyrazole/3-methylpyrazole/4-methylpyrazole/3,5-dimethylpyrazole, where M(II) = Co(II), Ni(II), Cu(II);
- M(II) : acrylic acid : imidazole/2-methylimidazole/5-methylimidazole/2-ethylimidazole, where M(II) = Co(II), Ni(II), Cu(II).

- M(II) : acrylic acid : benzimidazole/ 2-methylbenzimidazole/ 5-methylbenzimidazole/ 5,6-dimethylbenzimidazole, where M(II) = Co(II), Ni(II), Cu(II).

II.1. Complexes of Co(II), Ni(II) and Cu(II) with antimicrobial properties with acrylate ion and pyrazole/pyrazole derivatives as ligands

Complexes with mixed ligands (acrylate ion and pyrazole/pyrazole derivatives) were synthesized in two steps. In the first step were obtained the metal(II) acrylates by direct reaction between metal carbonates/hydroxycarbonates with acrylic acid in aqueous or alcoholic solution, afterwards was added pyrazole/pyrazole derivatives, in the molar ratio M(II): acrylic acid: pyrazole/pyrazole derivatives = 1:2:1; 1:2:2; 1:2:4. The synthesis lead to 16 new complexes, their formulations were proposed based on data obtained from elemental analysis, IR, UV-Vis-NIR and EPR spectra, magnetic measurements, thermal analysis and mass spectrometry, complexes were formulated as it follows:

$[Co(Hpz)_2(acr)_2]$	pink	**(1)**
$[Co(3\text{-}Mepz)_2(acr)_2]$	pink	**(2)**
$[Co(4\text{-}Mepz)_2(acr)_2]$	pink	**(3)**
$[Co(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)_2]\cdot 0{,}5H_2O$	pink	**(4)**
$[Co(3,5\text{-}Me_2pz)_2(acr)_2]$	violet	**(5)**
$[Ni(Hpz)_2(acr)_2(H_2O)]$	blue	**(6)**
$[Ni(3\text{-}Mepz)_2(acr)_2(H_2O)_2]\cdot 0{,}5\ H_2O$	blue	**(7)**
$[Ni(4\text{-}Mepz)_2(acr)_2(H_2O)_2]\cdot 6H_2O$	blue	**(8)**
$[Ni(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)]$	green	**(9)**
$[Cu_2(Hpz)_2(acr)_4]$	green	**(10)**
$[Cu(Hpz)_2(acr)_2(H_2O)]\cdot 1{,}5H_2O$	blue	**(11)**
$[Cu(Hpz)_2(acr)_2]$	purple	**(12)**
$[Cu(3\text{-}Mepz)_2(acr)_2(H_2O)]\cdot 1{,}5H_2O$	blue	**(13)**
$[Cu(4\text{-}Mepz)_2(acr)_2(H_2O)]\cdot H_2O$	blue	**(14)**
$[Cu_2(3,5\text{-}Me_2pz)_2(acr)_4]$	green	**(15)**
$[Cu(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)]\cdot 2H_2O$	blue	**(16)**

where Hpz ($C_3H_4N_2$) is pyrazole, 3-Mepz and 4-Mepz ($C_4H_6N_2$) are 3- and 4-methylpyrazole and 3,5-Me_2pz ($C_5H_8N_2$) is 3,5-dimethylpyrazole, and acr ($C_3H_3O_2$) is acrylate ion.

II.1.1. Synthesis and characterisation of some Co(II) complexes with antimicrobial properties with acrylate ion and pyrazole/pyrazole derivatives as ligands

There were obtained five new Co(II) complexes with acrylate ion and pyrazole/pyrazole derivatives, following the systems:

- Co(II) acrylate:pyrazole	(1:2)	$[Co(Hpz)_2(acr)_2]$ (**1**)
- Co(II) acrylate:3-methylpyrazole	(1:2)	$[Co(3\text{-}Mepz)_2(acr)_2]$ (**2**)
- Co(II) acrylate:4-methylpyrazole	(1:2)	$[Co(4\text{-}Mepz)_2(acr)_2]$ (**3**)
- Co(II) acrylate:3,5-dimethylpyrazole	(1:2)	$[Co(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)_2]\cdot 0{,}5H_2O$ (**4**)
	(1:4)	$[Co(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)_2]$ (**5**)

II.1.1.1. UV-Vis-NIR spectra and magnetic measurements

UV-Vis-NIR spectra correlated with magnetic moments indicated the complexes stereochemistries:

$[Co(Hpz)_2(acr)_2]$ **(1)**	Octahedral tetragonally distorted
$[Co(3\text{-}Mepz)_2(acr)_2]$ **(2)**	Octahedral tetragonally distorted
$[Co(4\text{-}Mepz)_2(acr)_2]$ **(3)**	Octahedral tetragonally distorted
$[Co(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)_2]\cdot 0,5H_2O$ **(4)**	Octahedral tetragonally distorted
$[Co(3,5\text{-}Me_2pz)_2(acr)_2]$ **(5)**	tetrahedral

II.1.1.2. IR spectra

Based on IR spectral data, there can be obtained the following informations:

- The presence of acrylate ion and pyrazole/pyrazole derivatives in all five complexes;
- The ligands are coordinated as it follows:

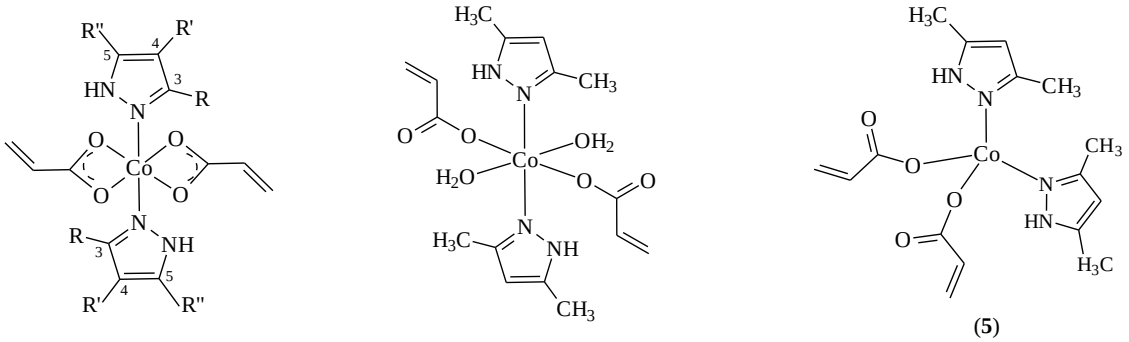
$[Co(Hpz)_2(acr)_2]$ **(1)**	- coordination through imine nitrogen atoms of pyrazole ligands unidentate coordinated; - coordination through oxygen atoms from acrylate ions bidentate coordinated;
$[Co(3\text{-}Mepz)_2(acr)_2]$ **(2)**	- coordination through imine nitrogen atoms of 3-methylpyrazole ligands unidentate coordinated; - coordination through oxygen atoms from acrylate ions bidentate coordinated;
$[Co(4\text{-}Mepz)_2(acr)_2]$ **(3)**	- coordination through imine nitrogen atoms of 4-methylpyrazole ligands unidentate coordinated; - coordination through oxygen atoms from acrylate ions bidentate coordinated;
$[Co(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)_2]\cdot 0,5H_2O$ **(4)**	- coordination through imine nitrogen atoms of 3,5-dimethylpyrazole ligands unidentate coordinated; - coordination through oxygen atoms from acrylate ions unidentate coordinated; - coordination through oxygen atoms from two coordinated water molecules;
$[Co(3,5\text{-}Me_2pz)_2(acr)_2]$ **(5)**	- coordination through imine nitrogen atoms of 3,5-dimethylpyrazole ligands unidentate coordinated; - coordination through oxygen atoms from acrylate ions unidentate coordinated.

II.1.1.3. Thermal analysis

Data provided by thermal analysis of all five Co(II) complexes confirmed the presence of acrylate ion and pyrazole/pyrazole dervatives, as well as the presence of water molecules in complexes (**4**) and (**5**).

II.1.1.4. Proposed formulation

Based on data provided by chemical analysis, thermal analysis, IR and UV-Vis-NIR spectra there were proposed the following formulations for complexes (**1**)-(**5**):



(**1**) R:H; R':H; R":H
(**2**) R:CH_3; R':H; R":H
(**3**) R:H; R':CH_3; R":H

(**4**)

(**5**)

Fig.II.1.1.6. Proposed formulations for complexes (**1**)-(**5**)

II.1.1.5. Biological activity tests

Determination of minimal inhibitory concentration(MIC)

Complexes (**4**) and (**5**) presented good activity [241] against the bacterial strain *Bacillus subtillis* ATCC 6633, with a MIC value of 125 μg/mL.

Influence on microbial biofilm developing on inert substrate

This study results revealed the fact that at concentrations higher than 125 μg/mL the complexes (**4**) and (**5**) present an inhibitory effect on adherence at inert substrate against the bacterial strain *Bacillus subtillis* ATCC 6633, but also at concentrations lower than 125 μg/mL it can be it can be talk about an opposite effect, stimulation of the microbial aherence on nert substrate [241].

Cytotoxicity studies

Cytotoxicity evaluation *in vitro* of the new synthesized compounds represent a fundamental stage to establish safety and applicability of the products. Cells culture represents an important way for preliminary evaluation of the new synthesized compounds cytotoxicity [243].

It was compared the effect of the complexes (**4**) and (**5**) on HCT8ccells culture. Cells were treated with concentrations in the range 12,5 - 200 μg/ml. Dose-effect curves revealed that one day treatement with more than 50 μg/ml degreases severely the cellular viability.

II.1.2. Synthesis and characterisation of some Ni(II) complexes with antimicrobial properties with acrylate ion and pyrazole/pyrazole dervatives ligands

There were obtained four new complexes of Ni(II) with acrylate and pyrazole/pyrazole derivatives as ligands, from the following systems:

- Ni(II) acrylate:pyrazole (1:2) $[Ni(Hpz)_2(acr)_2(H_2O)]$ (**6**)
- Ni(II) acrylate:3-methylpyrazole (1:2) $[Ni(3\text{-}Mepz)_2(acr)_2(H_2O)_2]\cdot 0{,}5\ H_2O$ (**7**)
- Ni(II) acrylate:4-methylpyrazole (1:2) $[Ni(4\text{-}Mepz)_2(acr)_2(H_2O)_2]\cdot 6H_2O$ (**8**)
- Ni(II) acrylate:3,5-dimethylpyrazole (1:2) $[Ni(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)]$ (**9**).

The experimental techniques used in characterisation of these new complexes to establish thier composition and chemical structure are chemical analysis, IR and UV-Vis-NIR spectra, magnetic measurements, mass spectra and thermal analysis.

II.1.2.1. UV-Vis-NIR spectra and magnetic measurements

UV-Vis-NIR spectra correlated with magnetic measurements indicated the octahedral tetragonally distorted geometry [230, 234] of Ni(II) complexes with acrylate ion and pyrazole/pyrazole derivatives.

II.1.2.2. IR spectra

IR spectra of the complexes (**6**)-(**9**) give the following informations [235]:

- Presence of acrylate ion and pyrazole/pyrazole derivatives ligands in all four complexes;
- Presence of water molecules in complexes composition;
- Acrylate ion coordination mode is unidentate and bidentate chelate in complexes (**6**) and (**9**), and unidentate in complexes (**7**) and (**8**).

II.1.2.3. Thermal analysis

Data provided by thermal analysis provided next informations:

- Presence of acrylate ion and pyrazole/pyrazole derivatives in all four complexes composition;
- Presence, nature and number of water molecules in the composition of all complexes discused in this chapter.

II.1.2.4. Mass spectrometry

Mass spectra recorded for compounds (**6**)-(**9**) confirmed their proposed formulations, revealing the presence of characteristic peaks of the fragments resulted after the ionization and framnetation of molecular ions [246].

II.1.2.5. Proposed formulations

On the basis of data provided by chemical analysis, thermal analysis, IR and UV-Vis-NIR spectra and mass spectra the complexes (**6**)-(**9**) were formulated as follows:

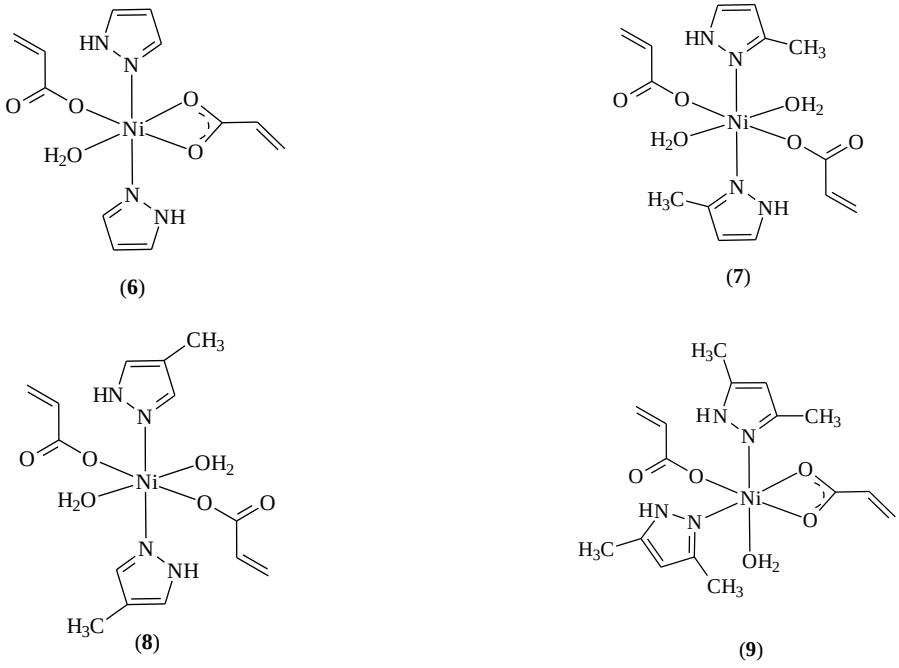


Fig.II.1.2.8. Proposed formualtions for complexes (**6**)-(**9**)

II.1.2.6. Biological activity tests

Determination of minimal inhibitory concentration (MIC)

The quantitative study in order to determine the minimal inhibitory concentration [241] revealed the moderate antimicrobial activity of complexes (**7**) and (**9**) against *E. faecium*, *E. coli* and *C. albicans* with a MIC value of 500 μg/mL.

Influence on microbial biofilm developing on inert substrate

The study concerning the complexes influence on microbial biofilm developing on inert substrate [241] revealed the fact that the same two complexes (**7**) and (**9**) present an inhibitory effect against adherence on inert substrate of the bacterial strains *E. faecium* and *B. subtillis*.

Cytotoxicity studies

It was observed that at low concentrations of tested substances the cellular viability is not affected, but with concentration increasing (over 50 μg/mL) it is observed a drastically damage on cellular viability [243].

II.1.3. Synthesis and characterisation of some Cu(II) complexes with antimicrobial activity with acrylate ion and pzrayole/pyrazole derivatives

There were obtained seven new complexes of Cu(II) with acrylate ion and pyrazole/pyrazole derivatives, from the systems:

- acrylate de Cu(II):pyrazole (1:1) $[Cu_2(Hpz)_2(acr)_4]$ (**10**);
  (1:2) $[Cu(Hpz)_2(acr)_2(H_2O)]\cdot 1{,}5H_2O$ (**11**);
  (1:2) $[Cu(Hpz)_2(acr)_2]$ (**12**);
- acrylate de Cu(II):3-methylpyrazole (1:2) $[Cu(3\text{-}Mepz)_2(acr)_2(H_2O)]\cdot 1{,}5H_2O$ (**13**);
- acrylate de Cu(II):4-methylpyrazole (1:2) $[Cu(4\text{-}Mepz)_2(acr)_2(H_2O)]\cdot H_2O$ (**14**);
- acrylate de Cu(II):3,5-dimethylpyrazole (1:1) $[Cu_2(3,5\text{-}Me_2pz)_2(acr)_4]$ (**15**);
  (1:2) $[Cu(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)]\cdot 2H_2O$ (**16**);

The factors that influenced the isolated species diversity from a certain system were:

- molar ratio of the components;
- nature of the solvent used in the synthesis;
- nature of the solvent used for recrystallization.

II.1.3.1. UV-Vis-NIR spectra and spectre EPR

UV-Vis-NIR correlated with EPR spectra confirmed the mantaining of the copper(II) oxidation state [230, 238] and indicated the following stereochemistries for the seven complexes:

$[Cu_2(Hpz)_2(acr)_4]$ **(10)**	Square pyramidal	
$[Cu(Hpz)_2(acr)_2(H_2O)]\cdot 1{,}5H_2O$ **(11)**	Square pyramidal	
$[Cu(Hpz)_2(acr)_2]$ **(12)**	Octahedral distorted	tetragonally
$[Cu(3\text{-}Mepz)_2(acr)_2(H_2O)]\cdot 1{,}5H_2O$ **(13)**	Square pyramidal	
$[Cu(4\text{-}Mepz)_2(acr)_2(H_2O)]\cdot H_2O$ **(14)**	Square pyramidal	
$[Cu_2(3,5\text{-}Me_2pz)_2(acr)_4]$ **(15)**	Square pyramidal	
$[Cu(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)]\cdot 2H_2O$ **(16)**	Square pyramidal	

II.1.3.2. IR spectra

The most important informations obtained from IR specta are [235]:

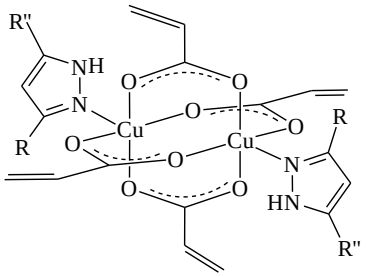
- presence of acrylate ion and pyrazole/pyrazole derivatives as ligands;
- water molecule presence in composition of complexes (**11**), (**13**), (**14**) and (**16**);
- the coordination mode of acrylate ion is bidentate in complexes (**10**), (**12**) and (**15**), and unidentate in complexes (**11**), (**13**), (**14**) and (**16**).

II.1.3.3. Thermal analysis

Data provided by thermal analysis revealed the tehrmal stability of the anhydrous complexes (**10**), (**12**) and (**15**) and the presence of water molecules in the composition of complexes (**11**), (**13**), (**14**) and (**16**).

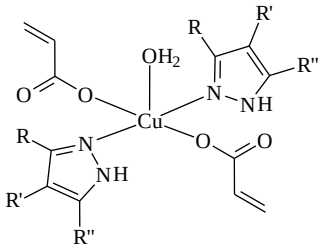
II.1.3.5. Proposed formulations

Based on data provided by chemical analysis, IR and UV-Vis-NIR and thermal analysis complexes (**10**)-(**16**) were formulated as it follows.



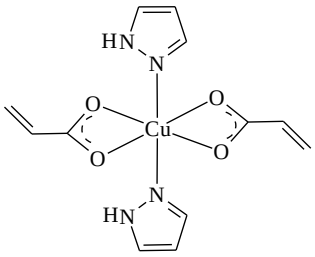
(**10**) R:H; R":H
(**15**) R:CH_3; R":CH_3

Fig.II.1.3.15. Proposed formulations for complexes (**10**) and (**15**)



(**11**) R:H; R':H; R":H
(**13**) R:CH_3; R':H; R":H
(**14**) R:H; R':CH_3; R":H
(**16**) R:CH_3; R':H; R":CH_3

Fig.II.1.3.16. Proposed formulations for complexes (**11**), (**13**), (**14**) and (**16**)



(**12**)

Fig.II.1.3.17. Proposed formulation for complexe (**12**)

II.1.3.6. Biological activity tests

Determination of minimal inhibitory concentration (MIC)

The antimicrobial activity was tested for all the compounds against the bacterial strains *E. faecium E5, E. coli ATCC 25922, C. albicans 1760, P. aeruginosa 27857, B. subtillis ATCC 6538, S. aureus ATCC 6538 and K. penumoniae IC 13420*, and it was observed the good antifungal activity with MIC value of 125 μg/mL and good antimicrobial activity against *E. coli* with MIC value of 250 μg/mL.

Influence on microbial biofilm developing on inert substrate

The study concerning the influence of the complexes on the microbial adherence on inert substrate [241] revealed that:

- Complexes (**12**) and (**13**) present an inhibitory effect against *E. faecium* and *S. aureus* at MIC values higher than 250 μg/mL;
- Complex (**16**) presents an inhibitory effect against *E. faecium* and *B. subtillis* at MIC values higher tahn 125 μg/mL and against *S. aureus* for MIC values higher than 250 μg/mL.

Cytotoxicity studies

The cell cycle analysis [243] in the presence of 100 μg/ml of compound (**12**), (**13**), (**16**) showed that these susbtances produce an acumulation of the cells in the phase G2/M, so they can be considered as mitotic blocking agents, this results indicating the fact that they can be further studied as cytostatic agents.

Studies of anti-inflammatory activity

Complex (**16**) was evaluated for the posible anti-inflammatory properties [249], and it was observed a very good antiinflammatory effect, comparable with that of the diclofenac drug, which was used as reference drug.

II.2. Complexes of Co(II), Ni(II) and Cu(II) with antimicrobial properties with acrylate and imidazole/imidazole derivatves as ligands

Complexes with mixed ligands (acrylate ion and pyrazole/pyrazole derivatives) were synthesized in two steps. In the first step were obtained the metal(II) acrylates by direct reaction between carbonates/hidroxycarbonates with acrylic acid în aqueous or alcoholic solution, afterwards was added pyrazole/pyrazole derivatives, in the molar ratio M(II): acrylic acid: imidazole/imidazole derivatives = 1:2:2; 1:2:4.

Based on data provided by elemental analysis, IR, UV-Vis-NIR and EPR spectra, thermal analysis, magnetic measurements, the new synthesized complexes were formulated as it follows:

$[Co(HIm)_2(acr)_2] \cdot 2{,}5\ H_2O$	violet	**(17)**
$[Co(2\text{-}MeIm)_2(acr)_2]$	violet	**(18)**
$[Co(5\text{-}MeIm)_2(acr)_2]$	violet	**(19)**
$[Co(2\text{-}EtIm)_2(acr)_2]$	violet	**(20)**
$[Ni(HIm)_4(acr)_2]\cdot 0{,}5\ H_2O$	purple	**(21)**
$[Ni(HIm)_2(acr)_2]$	blue	**(22)**
$[Ni(2\text{-}MeIm)_2(acr)_2(H_2O)] \cdot H_2O$	green	**(23)**
$[Ni(5\text{-}MeIm)_2(acr)_2] \cdot H_2O$	blue	**(24)**
$[Ni(2\text{-}EtIm)_2(acr)_2(H_2O)] \cdot H_2O$	green	**(25)**

$[Cu(2\text{-}MeIm)_2(acr)_2] \cdot 2\ H_2O$	blue	**(26)**
$[Cu(2\text{-}MeIm)_2(acr)_2] \cdot 0{,}5\ H_2O$	purple	**(27)**
$[Cu(5\text{-}MeIm)_2(acr)_2] \cdot 0{,}5\ H_2O$	purple	**(28)**
$[Cu(2\text{-}EtIm)_2(acr)_2] \cdot 4\ H_2O$	blue	**(29)**
$[Cu(2\text{-}EtIm)_2(acr)_2]$	purple	**(30)**

where HIm ($C_3H_4N_2$) is imidazole, 2-MeIm and 5-MeIm ($C_4H_6N_2$) are 2- and 5-methylimidazole, and 2-EtIm ($C_5H_8N_2$) is 2-ethylimidazole and acr ($C_3H_3O_2$) is acrylate ion.

II.2.1. Synthesis and characterisation of some Co(II) complexes with antimicrobial properties with acrylate ion and imidazole/imidazole derivatives as ligands

It was synthesized a number of four new complexes of Co(II) with acrylate ion and imidazole/imidazole derivatives following the systems:

- Co(II) acrylate:imidazole (1:2) $[Co(HIm)_2(acr)_2] \cdot 2{,}5H_2O$ (**17**)
- Co(II) acrylate:2-methylimidazole (1:2) $[Co(2\text{-}MeIm)_2(acr)_2]$ (**18**)
- Co(II) acrylate:5-methylimidazole (1:2) $[Co(5\text{-}MeIm)_2(acr)_2]$ (**19**)
- Co(II) acrylate:2-ethylimidazole (1:2) $[Co(2\text{-}EtIm)(acr)_2]$ (**20**);

From all the approached systems it was obtained only one violet compound for each ligand, the molar ratio Co(II):acrylic acid: imidazole/imidazole derivatives was in all four cases 1:2:2.

II.2.1.1. UV-Vis-NIR spectra and magnetic measurements

UV-Vis-NIR spectra [230] of all these complexes correlated with magnetic measurements [234] indicate the distorted tetrahedral stereochemistry of the Co(II) ion.

II.2.1.2. IR spectra

IR spectra analysis [235] results in:

- the presence of acrylate ion and imidazole/imidazole derivatives as ligands;
- the coordination mode of the acrylate ligands is unidentate în toate combinațiile complexe (**17**)-(**20**);
- the presence of water molecules in composition of complex (**17**).

II.2.1.3. Thermal analysis

Thermal analysis confirmed the water molecules in complex (**17**) composition and the anhydrous nature of complexes (**18**) - (**20**) and the thermal stability for these anhydrous ones .

II.2.1.4. X-ray diffraction and proposed formulations

With the eception of complex (**18**) which was obtained as appropiate single-crystals for X-ray diffraction, the other complexes were formulated on the basis of data provided by chemical analysis, thermal analysis and IR and UV-Vis-NIR spectra.

Sinle-crystal X-ray diffraction analysis performed on complex (**18**) revealed the tetrahedral stereochemistry of the Co(II) ion. The 2-methylimidazole ligands are unidentate coordinated through their iminic nitrogen atoms. Acrylate coordination mode is unidentate, through oxygen atoms of the carboxylic group. Due to the different nature of the ligands, the tetrahedral stereochemistry is easy distorted, fact revealed in different bond lenghts and angles [252].

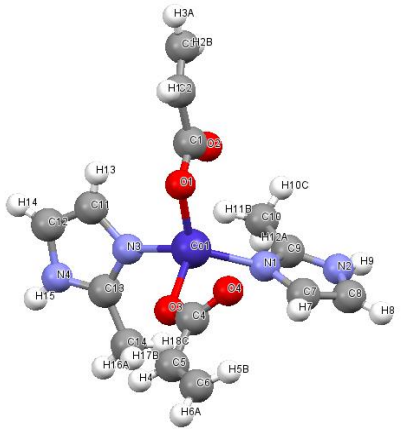
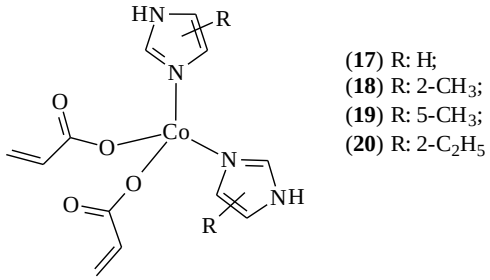


Fig.II.2.1.6. Crystal structure for complex (**18**)

On the basis of structural similarities between the Co(II) complexes, the best formulation (figure II.2.1.9) is the same as that established through X-ray diffraction for complex (**18**), for all four complexes the chromophore is $[CoN_2O_2]$.



(**17**) R: H;
(**18**) R: 2-CH_3;
(**19**) R: 5-CH_3;
(**20**) R: 2-C_2H_5

Fig.II.2.1.9. Proposed formulations for complexes (**17**), (**18**), (**19**) and (**20**)

II.2.1.5. Biological activity tests

Determination of minimal inhibitory concentration (MIC)

Biological tests were performed against bacterial strains, Gram-positive *E. faecium* and *B. subtillis*, Gram-negative *E. coli* and *S. aureus*, and fungal strain *C. albicans* [241]. The results revealed a good and very good antimicrobial activity of these complexes (**17**)-(**20**) with MIC values in the range 31,25 - 125 μg/mL against all the tested microbial strains.

Influence on microbial biofilm developing on inert substrate

It was observed that all complexes present an inhibitory effect on the microbial adherence on inert substrate [241] at concentrations higher than 31,25 μg/mL.

Cytotoxicity studies

It was found that these complexes (**17**)-(**20**) have a similar effect, presenting a low cytotoxic effect, HCT8 cells viability [243] being less affected at concentrations hgher than 100 μg/ml, these compounds being further studied as antimicrobial agents.

II.2.2. Synthesis and characterisation of some Ni(II) complexes with antimicrobial properties with acrylate and imidazole/imidazole derivatives as ligands

There synthesized five new Ni(II) complexes with acrylate ion and imidazole/imidazole derivatives as ligands, following the systems:

- Ni(II) acrylate:imidazole (1:4) $[Ni(HIm)_4(acr)_2]\cdot 0{,}5H_2O$ (**21**)
  (1:2) $[Ni(HIm)_2(acr)_2]$ (**22**)
- Ni(II) acrylate:2-methylimidazole (1:2) $[Ni(2\text{-}MeIm)_2(acr)_2(H_2O)]\cdot H_2O$ (**23**)
- Ni(II) acrylate:5-methylimidazole (1:2) $[Ni(5\text{-}MeIm)_2(acr)_2]\cdot H_2O$ (**24**)
- Ni(II) acrylate:2-ethylimidazole (1:2) $[Ni(2\text{-}EtIm)_2(acr)_2(H_2O)]\cdot H_2O$ (**25**).

II.2.2.1. UV-Vis-NIR spectra and magnetic measurements

Data provided by UV-Vis-NIR spectra and magnetic measurements [230, 234] can be concluded as it follows:

- In all five complexes the Ni(II) ion stereochemistry is octahedral tetragonally distorted;
- It could be observed following the absorbtion bands intensities the ligands position, cis or trans; so, complexes (**21**)-(**24**) have a cis conformation and the complex (**25**) has a trans conformation.

II.2.2.2. IR spectra

Conclusions that can be formulated based on IR spectral data are:

- The presence of acrylate ion and imidazole derivatives as ligands;
- The coordination water molecules in complexes (**23**) and (**25**);
- Acrylate ion coordination mode [235] is:
  - In complex (**21**), unidentate;
  - In complex (**22**) and (**24**), bidentate;
  - In complexes (**23**) and (**25**), unidentate and bidentate, simultaneously.

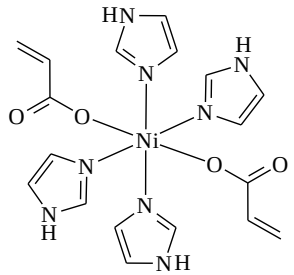
II.2.2.3. Thermal analysis

Thermal behaviour data of the complexes (**21**)-(**25**) provided the next informations:

- presence of acrylate ion and imidazole/imidazole derivatives ligands in all five complexes composition;
- water molecule presence in complexes (**21**), (**23**), (**24**) and (**25**);
- thermal stability of the anhydrous compounds resulted after elimination of water molecules in the frst step of thermal decomposition.

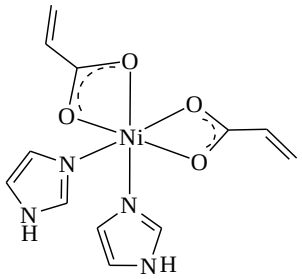
II.2.2.4. Proposed formulation

Complexes (**21**)-(**25**) were formulated based on results obtained from chemical analysis, thermal analysis, IR and UV-Vis-NIR spectra, as it can be observed in figures II.2.2.7 and II.2.2.8.

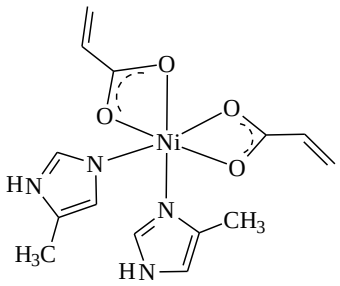


(**21**)

Fig.II.2.2.7. Proposed formulation for (**21**)

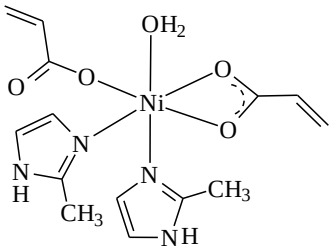


(**22**)

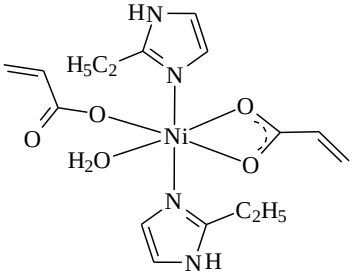


(**24**)

Fig.II.2.2.8. Proposed formulations for (**22**) and (**24**)



(**23**)



(**25**)

Fig.II.2.2.9. Proposed formulations for (**23**) and (**25**)

II.2.2.5. Biological activity tests

Determination of minimal inhibitory concentration(MIC)

Complex $[Ni(HIm)_2(acr)_2]$ (**22**) presented moderate antimicrobial activity against *E. faecium* with MIC value of 250 μg/mL. The complex $[Ni(2\text{-}MeIm)_2(acr)_2(H_2O)]\cdot H_2O$ (**23**) presented moderate antimicrobial activity against *B. subtillis* and *P. aeruginosa* with MIC value of 250 μg/mL.

Influence on microbial biofilm developing on inert substrate

It was evidenced an inhibitory effect on microbial adherence to inert substrate [241] for complexes (**22**)-(**24**) against bacterial strains *E. faecium, E. coli, B. subtillis* and *K. pneumoniae*.

II.2.3. Synthesis and characterisation of some Cu(II) complexes with antimicrobial properties with acrylate ion and imidazole derivatives

There were isolated and characterised five new Cu(II) complexes with acrylate and imidazole derivatives ligands, following the next systems:

- copper(II) acrylate:2-methylimidazole (1:2) $[Cu(2\text{-}MeIm)_2(acr)_2]\cdot 2H_2O$ (**26**)
  $[Cu(2\text{-}MeIm)_2(acr)_2]\cdot 0{,}5H_2O$ (**27**)
- copper(II) acrylate:5-methylimidazole (1:2) $[Cu(5\text{-}MeIm)_2(acr)_2]\cdot 0{,}5\ H_2O$ (**28**)
- copper(II) acrylate:2-ethylimidazole (1:2) $[Cu(2\text{-}EtIm)_2(acr)_2]\cdot 4H_2O$ (**29**)
  $[Cu(2\text{-}EtIm)_2(acr)_2]$ (**30**);

II.2.3.1. UV-Vis-NIR and EPR spectra

UV-Vis-NIR spectra correlated with EPR spectra [230, 238] confirmed the oxidation state of Cu(II) ion and the octahedral tetragonally distorted stereochemistry in all five complexes.

II.2.3.2. IR spectra

IR spectra analysis revealed:

- the presence of acrylate and imidazole derivatives in all complexes;
- the presence of water crystallization molecules in complexes (**26**), (**27**), (**28**) and (**29**);
- the bidentate coordination mode of acrylate ions in all five complexes [235].

II.2.3.3. Thermal analysis

Thermal analysis data revealed the similar thermal behaviour of the blue coloured complexes (**26**) and (**29**), otherwise the similar thermal behaviour of the purple coloured complexes(**27**), (**28**) and (**30**). The hydrated nature of the compounds (**26**) - (**29**) and the hydrated nature of the compound (**30**) were confirmed.

II.2.3.4. X-ray diffraction and proposed formulation

Based on single crystal X-ray diffraction analysis it was determined the crystal structure for complexes (**27**), (**29**) and (**30**).

The compound (**27**) has an octahedral stereochemistry, the copper(II) ion is coordinated by two bidentate acrylate ions and two unidentate 2-methylimidazole molecules. The two 2-methylimidazole ligands are trans positioned. The compound is monomeric with a centrosymmetric structure, the CuO_4 unity in the ecuatorial plane being perfectly coplanar (figura II.2.3.8) [252].

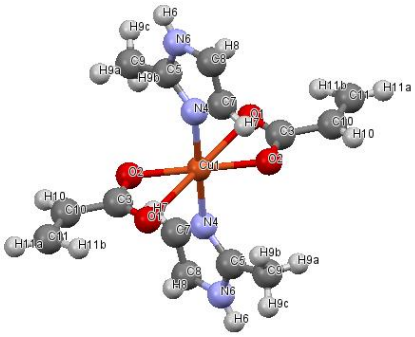


Fig.II.2.3.8. Crystal structure of $[Cu(2\text{-}MeIm)_2(acr)_2]$ (**27**)

The compound (**29**) is monomeric, the copper(II) ion being in an octahedral tetragonally distorted stereochemistry with the cis-CuN_2O_4 chromophore. Because of the cis position of the ligands, the octahedral stereochemistry is strongly distorted so that it can be confused with trigonal prism.

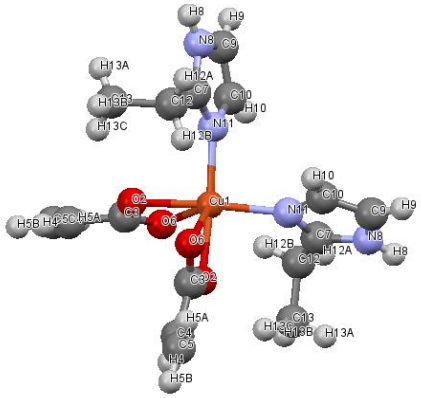


Fig.II.2.3.11. Crystal structure of $[Cu(2\text{-}EtIm)_2(acr)_2] \cdot 4H_2O$ (**29**)

Complex (**30**) has an octahedral sterochemistry, the copper(II) ion is coordinated to two acrylate ions that are bidentate ligands and to two molecules of 2-ethylmidazole, these ligands being in trans positions. This complexe is monomeric with centrosymmetric structure, the CuO_4 unity from ecuatorial plane being perfectly coplanar [252].

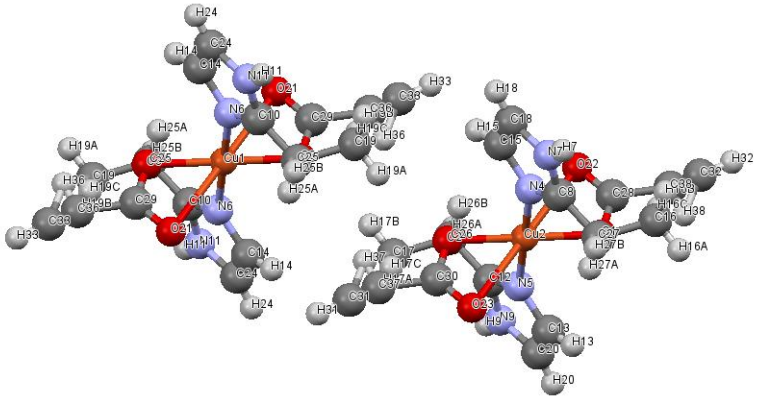
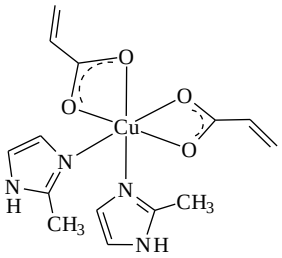
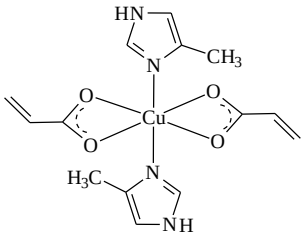


Fig.II.2.3.15. Crystal structure of complex $[Cu(2\text{-}EtIm)_2(acr)_2]$ (**30**)

For the complexes (**26**) and (**28**) which were obtained only as crystalline powders, were proposed the following formulations:



(**26**)



(**28**)

Fig.II.2.3.18. Proposed fromulations for complexes (**26**)

II.2.3.5. Biological activity tests

Determination of minimal inhibitory concentration (MIC)

It was observed that the only compound that presentd good antimicrobial activity with MIC value of 125 µg/mL against the bacterial strains *E. faecium*, *E. coli*, *C. albicans*, *B. subtillis* and *S. aureus* is the compound $[Cu(2\text{-}MeIm)_2(acr)_2]\cdot 2H_2O$ (**26**).

Influence on microbial biofilm developing on inert substrate

The study concerning the microbial biofilm developing on inert substrate revealed the fact that only the compound $[Cu(2\text{-}MeIm)_2(acr)_2]\cdot 2H_2O$ (**26**) presents a considerable inhibitory effect against the adherence on inert substrate [241].

Cytotoxicity studies

The tested compounds, (**26**) and (**28**) had no effect on the cells viability, only at high concentrations of over 200 µg/ml [243], thing that can make them good candidates for further utilisation as antimicrobial agents.

II.3. Complexes of Co(II), Ni(II) and Cu(II) with antimicrobial properties with acrylate ion and benzimidazole/benzimidazole derivatives as ligands

Complexes with mixt ligands (acrylate ion and benzimidazole/benzimidazole derivatives) were synthesized in two steps. First it was obtained the metal(II) acrylate by direct reaction between metal carbonates/hydroxycarbonates with acrylic acid in aqueous or alcoholic solution afterwards was added the benzimidazole/benzimidazole derivative. Molar ratios used in the synthesis of these complexes of Co(II), Ni(II), Cu(II) were: M(II): acrylic acid: benzimidazole/benzimidazole derivative = 1:2:1; 1:2:2.

On the basis of data provided by elemental analysis, IR, UV-Vis-NIR and RES spectra, thermal analysis, magnetic measurements, the new synthesized complexes were formulated as it follows:

$[Co(HBzIm)_2(acr)_2] \cdot 0,5\ H_2O$	violet	**(31)**
$[Co(2\text{-}MeBzIm)_2(acr)_2] \cdot 0,5\ H_2O$	violet	**(32)**
$[Co(5\text{-}MeBzIm)_2(acr)_2]$	violet	**(33)**
$[Co(5,6\text{-}Me_2BzIm)_2(acr)_2]$	violet	**(34)**
$[Ni(HBzIm)_2(acr)_2(H_2O)] \cdot 3\ H_2O$	blue	**(35)**
$[Ni(2\text{-}MeBzIm)_2(acr)_2(H_2O)] \cdot 1,5\ H_2O$	blue	**(36)**
$[Ni(5\text{-}MeBzIm)_2(acr)_2(H_2O)]$	blue	**(37)**
$[Ni(5,6\text{-}Me_2BzIm)_2(acr)_2]$	blue	**(38)**
$[Cu(BzIm)_2(H_2O)] \cdot H_2O$	red	**(39)**
$[Cu_2(HBzIm)_2(acr)_4]$	green	**(40)**
$[Cu(HBzIm)_2(acr)_2]$	blue	**(41)**
$[Cu(HBzIm)_2(acr)_2(H_2O)] \cdot H_2O$	purple	**(42)**
$[Cu_2(2\text{-}MeBzIm)_2(acr)_4]$	green	**(43)**
$[Cu(2\text{-}MeBzIm)_2(acr)_2]$	purple	**(44)**
$[Cu_2(5\text{-}MeBzIm)_2(acr)_4]$	green	**(45)**
$[Cu(5\text{-}MeBzIm)_2(acr)_2(H_2O)] \cdot 2\ H_2O$	blue	**(46)**
$[Cu_2(5,6\text{-}Me_2BzIm)_2(acr)_4]$	green	**(47)**
$[Cu(5,6\text{-}Me_2BzIm)_2(acr)_2(H_2O)] \cdot 2\ H_2O$	blue	**(48)**
$[Cu(5,6\text{-}Me_2BzIm)_2(acr)_2]$	purple	**(49)**

where HBzIm ($C_7H_6N_2$) is benzimidazole, 2-MeBzIm and 5-MeBzIm ($C_8H_8N_2$) are 2- and 5-methylbenzimidazole, respectively, and 5,6-Me_2BzIm ($C_9H_{10}N_2$) is 5,6-dimethylbenzimidazole, and acr ($C_3H_3O_2$) is acrylate ion.

II.3.1. Synthesis and characterisation of some Co(II) complexes with antimicrobial properties with acrylate ion and benzimidazole/benzimidazole derivatives as ligands

There were synthesized and isolated four complexes of Co(II) wth acrylate and benzimidazole/benzimidazole derivatives as ligands, following the systems:

- Co(II) acrylate:benzimidazole (1:2) $[Co(HBzIm)_2(acr)_2] \cdot 0,5\ H_2O$ (**31**)
- Co(II) acrylate:2-methylbenzimidazole (1:2) $[Co(2\text{-}MeBzIm)_2(acr)_2] \cdot 0,5\ H_2O$ (**32**)
- Co(II) acrylate:5-methylbenzimidazole (1:2) $[Co(5\text{-}MeBzIm)_2(acr)_2]$ (**33**)
- Co(II) acrylate:5,6-dimethylbenzimidazole (1:2) $[Co(5,6\text{-}Me_2BzIm)_2(acr)_2]$ (**34**);

The experimental techniques used in formulation of these complexes are: chemical analysis, UV-Vis-NIR and IR spectra, magnetic measurements and thermal analysis.

II.3.1.1. UV-Vis-NIR spectra and magnetic measurements

Data obtained from UV-Vis-NIR spectra and magnetic measurements confirmed the distorted tetrahedral stereochemistry [230, 234] of the Co(II) ion in all four complexes (**31**)-(**34**).

II.3.1.2. IR spectra

From IR spectra analysis it was observed:

- The presence of acrylate ion and benzimidazole/benzimidazole derivatives ligands;
- Acrylate ion s unidentate coordinated [235] in all four Co(II) complexes;
- Water molecules presence in complexes (**31**) and (**32**).

II.3.1.3. Thermal analysis

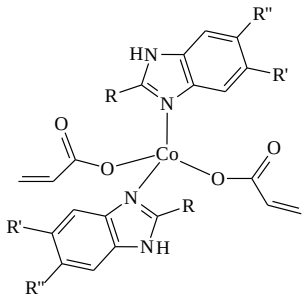
The results obtained from thermal analysis data:

- All four complexes contain acrylate ions and benzimidazole/benzimidazole derivatives ligands;
- Complexes (**31**) and (**32**) contain water molecules;
- Complexes (**33**) and (**34**) are anhydrous and very thermally stable, their decomposition is starting over 195 and 230 ºC, respectively.

II.3.1.4. Proposed formulations

Complexes of Co(II) with acrylate ion and benzimidazole/benzimidazole derivatives were formulated based on data provided by chemical analysis, thermal analysis, IR and UV-Vis-NIR spectra.

The structure proposed for these complexes is tetrahedral, two benzimidazoele ligands are unidentate coordinated in plane and two acrylate ions are unidentate coordinated in axial positions. In all four cases, the chromophore is $[CoN_2O_2]$.



(**31**) R:H; R':H; R":H
(**32**) R:CH_3; R':H; R":H
(**33**) R:H; R':CH_3; R":H
(**34**) R:H; R':CH_3; R":CH

Fig.II.3.1.9. Proposed formulations for complexes (**31**)-(**34**)

II.3.1.5. Biological activity tests

Determination of minimal inhibitory concentration (MIC)

The biological tests [241] were performed against the bacterial strains *S. aureus*, *B. subtillis*, *E. coli*, *E. faecium* and the fungal strain *C. albicans*, and it revealed a very good antimicrobial activity of these complexes with MIC values in the range 31,25 and 62,5 μg/mL.

Cytotoxicity studies

The cell cycle analysis of the HT29 cells treated with 100 μg/ml of complexes (**31**)-(**34**) showed the fact that these substances induce a consistent increasing of the G2/M faze of the cell cycle [243], this thing being interesting for further studies of these compounds as cytostatic agents.

II.3.2. Synthesis and characterisation of some Ni(II) complexes with antimicrobial properties with acrylate ion and benzimidazole/benzimidazole derivatives as ligands

There were isolated and characterised as crystalline powders four complexes of Ni(II) with acrylate ion and benzimidazole/benzimidazole derivatives as ligands, following the systems:

- Ni(II) acrylate:benzimidazole (1:2) $[Ni(HBzIm)_2(acr)_2(H_2O)]\cdot 3H_2O$ (**35**);
- Ni(II) acrylate:2-methylbenzimidazole (1:2) $[Ni(2\text{-}MeBzIm)_2(acr)_2(H_2O)]\cdot 1{,}5H_2O$ (**36**);
- Ni(II) acrylate:5-methylbenzimidazole (1:2) $[Ni(5\text{-}MeBzIm)_2(acr)_2(H_2O)]$ (**37**);
- Ni(II) acrylate:5,6-dimethylbenzimidazole (1:2) $[Ni(5,6\text{-}Me_2BzIm)_2(acr)_2]$ (**38**);

II.3.2.1. UV-Vis-NIR spectra and magnetic measurements

UV-Vis-NIR spectral data correlated with magnetic measurements [230, 234] provided the following informations:

- Ni(II) ion is in the same oxidation state in all four complexes;
- Ni(II) ion adopted the octahedral tetragonally distorted stereochemistry in all four complexes;
- Based on absorbtion bands position and on their instensities one can appreciate that in the complex (**35**) the benzimidazole ligands are placed in cis positions and in the other complexes (**36**)-(**38**) the benzimidazole derivatives ligands are placed in trans positions, taking in consideration the steric hindrance due to the susbtituents to the benzimidazole nucleus.

II.3.2.2. IR spectra

IR spectra analysis [235] provided informations like:

- presence in all complexes of acrylate ion and benzimidazole/benzimidazole derivatives as ligands;
- the acrylate ion coordination mode is both unidentate and bidentate in complexes (**35**), (**36**) and (**37**), and only bidentate in the case of complex (**38**);
- water molecule presence in the case of complexes (**35**), (**36**) and (**37**).

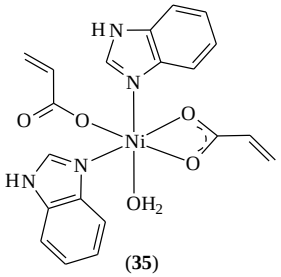
II.3.2.3. Thermal analysis

Thermal analysis provided the following data:

- water molecules presence in composition of complexes (**35**), (**36**) and (**37**);
- thermal stability of the anhydrous compounds, in all four cases the decomposition is starting over 250ºC.

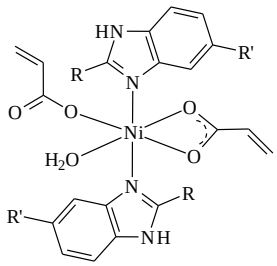
II.3.2.4. Proposed formulations

Based on data provided by different experimental techniques used in their characterisation were proposed the following formulations (**35**)-(**38**).



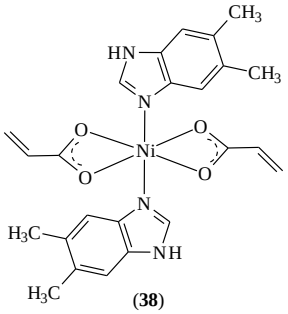
(**35**)

Fig.II.3.2.9. Proposed formulation for complex (**35**)



(**36**) R: CH_3; R': H;
(**37**) R: H; R': CH_3

Fig.II.3.2.10. Proposed formulation for complexes(**36**) and (**37**)



(**38**)

Fig.II.3.2.11. Proposed formulation for complex (**38**)

II.3.2.5. Biological activity tests

Determination of minimal inhibitory concentration (MIC)

Biological tests were performed against Gram-positive, *E. faecium*, *B. subtillis*, Gram-negative, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, bacterial strains, and against fungal strains *C. albicans*. The quatitative study [241] revealed the fact that all complexes (**35**) - (**38**) presented moderate antimicrobial activity with MIC values in the range 500-1000 μg/mL.

Influence on microbial biofilm developing on inert substrate

The study concerning the complexes influence on microbial biofilm developing on inert substrate revealed the fact that most of them presented an inhibitory effect at concentrations higher than 250 or 500 μg/mL.

II.3.3. Synthesis and characterisation of some Cu(II) complexes with acrylate ion and benzimidazole/benzimidazole derivatives

It was synthesized a number of 10 new complexes of (Cu) with acrylate and benzimidazole/benzmidazole derivatives as ligands, and one complex with benzimidazolate ion, following the systems [258]:

- copper(II) acrylate:benzimidazole	(1:1)	$[Cu(BzIm)_2(H_2O)]\cdot H_2O$ (**39**)
		$[Cu_2(HBzIm)_2(acr)_4]$ (**40**)
	(1:2)	$[Cu(HBzIm)_2(acr)_2]$ (**41**)
		$[Cu(HBzIm)_2(acr)_2]\cdot 1{,}5H_2O$ (**42**)
- copper(II) acrylate:2-methylbenzimidazole	(1:1)	$[Cu_2(2\text{-}MeBzIm)_2(acr)_4]$ (**43**)
	(1:2)	$[Cu(2\text{-}MeBzIm)_2(acr)_2]$ (**44**)
- copper(II) acrylate:5-methylbenzimidazole	(1:1)	$[Cu_2(5\text{-}MeBzIm)_2(acr)_4]$ (**45**)
	(1:2)	$[Cu(5\text{-}MeBzIm)_2(acr)_2(H_2O)]\cdot 2H_2O$ (**46**)
- copper(II) acrylate:5,6-dimethylbenzimidazole	(1:1)	$[Cu_2(5,6\text{-}Me_2BzIm)_2(acr)_4]$ (**47**)
	(1:2)	$[Cu(5,6\text{-}Me_2BzIm)_2(acr)_2(H_2O)]\cdot 2H_2O$ (**48**)
		$[Cu(5,6\text{-}Me_2BzIm)_2(acr)_2]$ (**49**)

II.3.3.1. UV-Vis-NIR and EPR spectra

The results obtained from UV-Vis-NIR and EPR spectral it was established the fact that the copper(II) ion has the following stereochemistries [230, 238]:

- square planar in complex (**39**), with chromophore [CuN_4];
- square pyramidal in binuclear complexes (**40**), (**43**), (**45**) and (**47**) , with chromophore [$CuNO_4$];
- square pyramidal in complexes (**41**), (**46**) and (**48**), with chromophore [CuN_2O_3];
- octahedral tetragonally distorted in complexes (**42**), (**44**) and (**49**), with chromophore [CuN_2O_4].

II.3.3.2. IR spectra

The comparison between IR spectra of complexes, sodium acrylate and benzimidazole/benzimidazole derivatives it was observed the following [235]:

- the absence of acrylate ion in complexe (**39**) and the presence of benzimidazole as anion;
- the presence of acrylate and benzimidazole/benzimidazole derivatives in complexes (**40**)-(**49**);
- the acrylate ion coordination mode is: unidentate in complexes (**46**) and (**48**); bridged bidentate in complexes (**40**), (**43**), (**45**) and (**47**); chelate bidentate in complexes (**42**), (**44**) and (**49**); unidentate and chelate bidentate in complex (**41**);
- water molecules presence in composition of complexes (**39**), (**42**), (**46**) and (**48**);
- benzimidazole/benzimidazole derivatives are coordinated through imine nitrogen atoms.

II.3.3.3. Thermal analysis

From thermal behaviour data there were revealed the following:

- Complexes composition;
- Anhydrous nature and the high thermal stability of the anhydrous complexes (**40**), (**41**), (**43**), (**44**), (**45**), (**47**) and (**49**);
- Presence of water molecules in complexes composition (**39**), (**42**), (**46**) and (**48**).

II.3.3.5. X-ray diffraction and proposed formulations

With the exception of complexes (**44**) and (**48**) which were obtained as appropiate single crystals for X-ray diffraction, all the other complexes were formulated based on data provided by chemical analysis, thermal analysis, IR, EPR and UV-Vis-NIR spectra.

Complexe (**44**) presents a monomeric structure and an octahedral geometry with chromophore trans-CuN_2O_4.

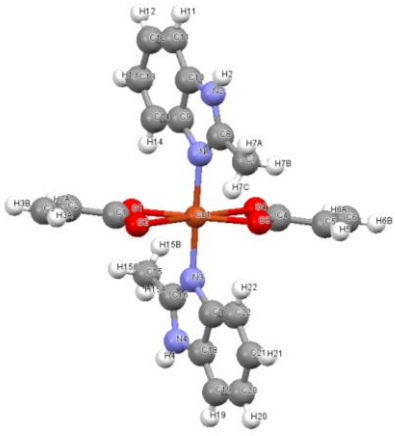


Fig.II.3.3.29. Crystal structure of complex (**44**)

The compound (**48**) presents a monomeric structure in which the copper(II) ion is pentacoordinated to two 5,6-dimethylbenzimidazole ligands, two acrylate ions and one water molecule, all the ligands are unidentate coordinated.

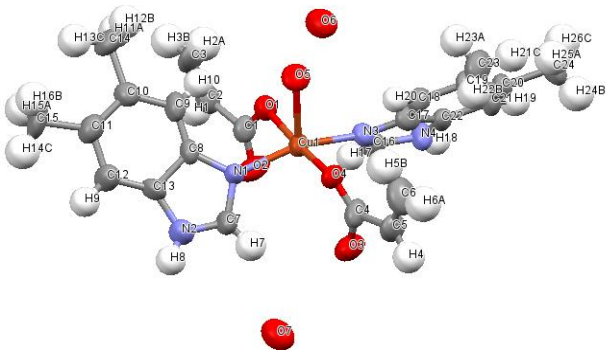
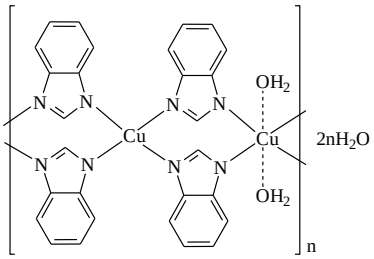


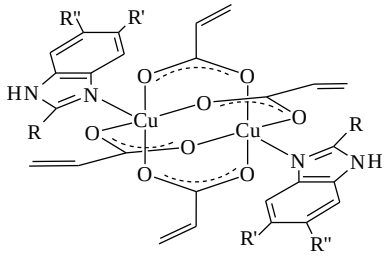
Fig.II.3.3.33. Crystal structure (**48**)

Complexes obtained only as crystalline powders were formulated based on experimetal data as it follows:



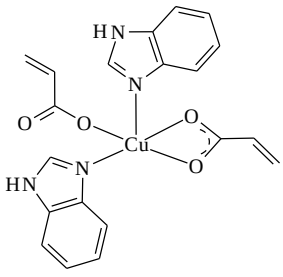
(39)

Fig.II.3.3.38. Proposed formulation for complex (**39**)



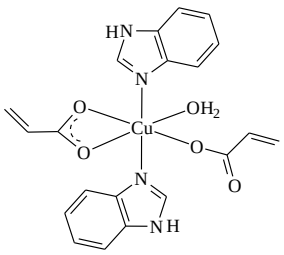
(**40**) R:H; R':H; R'':H
(**43**) R:CH_3; R':H; R'':H
(**45**) R:H; R':CH_3; R'':H
(**47**) R:H; R':CH_3; R'':CH_3

Fig.II.3.3.39. Proposed formulations for complexes (**40**), (**43**), (**45**) and (**47**)

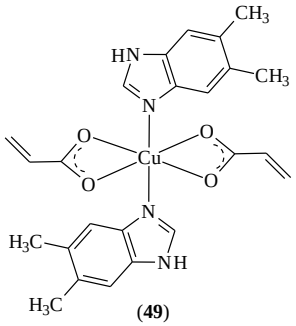


(**41**)

Fig.II.3.3.40. Proposed formulation for complex (**41**)

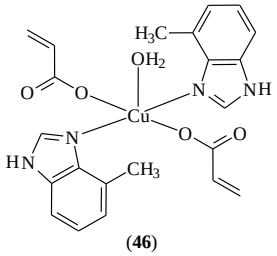


(**42**)



(**49**)

Fig.II.3.3.41. Proposed formulation (**42**) and (**49**)



(**46**)

Fig.II.3.3.42. Proposed formulation for complex (**46**)

II.3.3.6. Biological activity tests

The antimicrobial activity of the new compounds was evaluated against Gram-positive and Gram-negative bacterial strains, as well as against fungus strains [241].

Determination of minimal inhibitory concentration (MIC)

The biological tests revealed a very good, good and medium antimicrobial activity with broad spectrum, with MIC values in the range 31,25 - 250 μg/ml for all complexes described in this chapter aganst all microbial strains in the study.

Cytotoxicity studies

Cytotoxicity studies [243] revealed the low toxicity of the complexes (**39**), (**40**), (**42**), (**44**) and (**48**) (at concentrations higher than 100 μg/ml it drastically degreases the cellular viability) and the high toxicity of complexes (**41**), (**45**) and (**47**) (at concentrations higher than 50, 25 and 5 μg/ml, respectively, it drastically degrease the cellular viability).

Studies for anti-inflammatory activity

The antiinflammatory activity studies revealed the very good antiinflammatory effect of the compound (**48**), comparable to the diclofenac drug.

III. CONCLUSIONS

- There were synthesized, characterised and formulated **49** complexes that contain acrylic acid and azole type ligands: pyrazole/derivați de pyrazole, imidazole/derivați de imidazole and benzimidazole/benzimidazole derivatives;
- The system M(II) : acrylic acid : pyrazole/3-methylpyrazole/4-methylpyrazole/3,5-dimethylpyrazole, where M(II) = Co(II), Ni(II), Cu(II), resulted in 16 isolated and characterised complexes;
- The system M(II) : acrylic acid : imidazole/2-methylimidazole/5-methylimidazole/2-ethylimidazole, where M(II) = Co(II), Ni(II), Cu(II), resulted in 14 isolated and characterised complexes, four of them being analysed through single crystal X-ray diffraction;

- The system M(II) : acrylic acid : benzimidazole/2-methylbenzimidazole/5-methylbenzimidazole/5,6-dimethylbenzimidazole, where M^{n+} = Co^{2+}, Ni^{2+}, Cu^{2+}, resulted in 19 isolated and characterised complexes, two of them being analysed through single crystal X-ray diffraction;
- The compounds were characterised through chemical analysis, IR, UV-Vis-NIR, EPR spectroscopies, mass spectrometry, magnetic measurements, thermal analysis, cyclic voltammetry, single crystal X-ray diffraction.
- Thermal analysis identified the number and nature of water molecules (coordination or crystallisation water), thermal stability range and it was established the nature of the final residues of the thermal decomposition;
- The synthesis systems that contain acrylate ion and pyrazole/pyrazole derivatives lead to 16 complexes which were isolated, characterised and formulated on the basis of experimental data;
- From the synthesis systems that contain acrylate ion and imidazole/imidazole derivatives there were isolated and characterized 14 complexes, four of them were isolated as single crystals and characterized through single crystal X-ray diffraction;
- The systems that contain acrylate ion and benzimidazole/benzimidazole derivatives lead to 19 complexes, of which two complexes were isolated as single crystals and characterised through X-ray diffraction;
- Crystal structure of $[Co(2\text{-}MeIm)_2(acr)_2]$ (**18**) revealed Co(II) ion with tetrahedral stereochemistry, to which are unidentate coordinated two acrylate ions and two 2-methylimidazole ligands;
- Crystal structures of $[Cu(2\text{-}MeIm)_2(acr)_2]$ (**27**) and $[Cu(2\text{-}EtIm)_2(acr)_2]$ (**30**) are analogous, in which the copper(II) ion presents octahedral distorted stereochemistry, the two acrylate ions are bidentate coordinated and the two imidazole derivatives are in trans positions;
- The compounds $[Cu(2\text{-}EtIm)_2(acr)_2] \cdot 4H_2O$ (**29**) and $[Cu(2\text{-}EtIm)_2(acr)_2]$ (**30**) are conformational izomers, in the first one the two 2-ethylimidazole ligands are cis positioned and in the second one the two 2-ethylimidazole are trans positioned;
- The crystal structure of $[Cu(2\text{-}MeBzIm)_2(acr)_2]$ (**44**) is analogous to crystal structures of (**27**) and (**30**);
- Crystal structure of $[Cu(5,6\text{-}Me_2BzIm)_2(acr)_2(H_2O)] \cdot 2H_2O$ (**48**) presents copper(II) ion in a square pyramidal stereochemistry, the two acrylate ions and the two 5,6-dimethylbenzimidazole ligands are unidentate coordinated and a water molecule is coordinated in axial position;
- In the case of some systems with acrylate and azole type ligands (pyrazole/benzimidazole) in which Cu(II) ion is imiplicated it was observed the tendance to form more than one compounds, in which copper(II) ion is addopting different stereochemistries and acrylate ion presents different coordination modes;
- Complexes were obtained with good yields from the molar ratio metal acrylate: azole type ligands = 1:1 sau 1:2, as chemical analysis and thermal analysis confirmed;
- The thermal analisys concluded that among all complexes the most thermally stable are the cmplexes $[Co(3,5\text{-}Me_2pz)_2(acr)_2]$ (**5**) (210°C), $[Co(2\text{-}MeIm)_2(acr)_2]$ (**18**) (210°C), $[Co(2\text{-}EtIm)_2(acr)_2]$ (**20**) (268°C), $[Ni(HIm)_2(acr)_2]$ (**22**) (270°C), $[Co(5\text{-}MeBzIm)_2(acr)_2]$ (**33**) (230°C) and $[Ni(5,6\text{-}Me_2BzIm)_2(acr)_2]$ (**38**) (250°C), their decomposition starting over 210°C, this thing being a consequence of their anhydrous nature;
- For the Co(II) complexes with acrylate and pyrazole derivatives it was observed a good antimicrobial activity with MIC values of 125 μg/mL against *B. subtillis* for the complexes $[Co(3,5\text{-}Me_2pz)_2(acr)_2(H_2O)_2] \cdot 0{,}5H_2O$ (**4**) and $[Co(3,5\text{-}Me_2pz)_2(acr)_2]$ (**5**);
- For the system that contains Cu(II) complexes with acrylate and pyrazole derivatives it nwas observed a good antimicrobial activity of complex (**16**) with a MIC value of 125 μg/mL against *E. faecium* and *B. subtillis*;

- Co(II) complexes with acrylate and imidazole/imidazole derivatives (**17**)-(**20**) presented a very good and good acntimicrobial activity with MIC values in the range 31,25 - 125 µg/mL, against *E. faecium*, *S. aureus*, *B. subtillis*, *E. coli* and *C. albicans*, the complex $[Co(2\text{-}MeIm)_2(acr)_2]$ (**18**) being the most active;
- Among the Cu(II) complexes with acrylate and imidazole derivatives the most active was complex $[Cu(2\text{-}MeIm)_2(acr)_2] \cdot 2H_2O$ (**26**) with MIC value of 125 µg/mL against *E. faecium*, *E. coli*, *B. subtillis*, *S. aureus* and *C. albicans*.
- Co(II) complexes with acrylate and benzimidazole/benzimidazole derivatives presents a very good activity with MIC values between 31,25 and 62,5 µg/mL, against *E. faecium*, *E. coli*, *B. subtillis*, *S. aureus* and *C. albicans*;
- It was observed a very good antimicrobial activity with MIC values in the range 31,25-125 µg/mL for complexes $[Cu_2(HBzIm)_2(acr)_4]$ (**40**), $[Cu(HBzIm)_2(acr)_2(H_2O)] \cdot H_2O$ (**42**), $[Cu_2(2\text{-}MeBzIm)_2(acr)_4]$ (**43**), $[Cu(2\text{-}MeBzIm)_2(acr)_2]$ (**44**) and $[Cu(5,6\text{-}Me_2BzIm)_2(acr)_2(H_2O)] \cdot 2H_2O$ (**48**);
- Biological tests revealed the lower antimicrobial activity for Ni(II) complexes, this behaviour being explained by Ni(II) ion preference for octahedral stereochemistry. Otherwise, it was observed low antimicrobial activity for Cu(II) complexes with octahedral stereochemistry;
- The best antimicrobial activity was revealed for tetrahedral Co(II) complexes and for Cu(II) complexes with square pyramidal stereochemistry;
- Cytotoxicity studies revealed for the studied compounds:
  - high cytotoxicity for the pyrazole complexes, except the compounds $[Cu(Hpz)_2(acr)_2]$ (**12**) and $[Cu(3\text{-}Mepz)_2(acr)_2(H_2O)] \cdot 1{,}5H_2O$ (**13**) which presented low cytotoxicity;
  - low cytotoxicity for all imidazole bearing complexes;
  - low cytotoxicity of Co(II) complexes with benzimidazole derivatives, except compound $[Co(HBzIm)_2(acr)_2] \cdot 0{,}5\ H_2O$ (**31**) which presented high cytotoxicity;
  - copper(II) complexes with benzimidazole present low cytotoxicity, except compound $[Cu(HBzIm)_2(acr)_2]$ (**41**);
  - in the case of copper(II) complexes wth benzimidazole derivatives it was observed the high cytotoxicity of $[Cu_2(5\text{-}MeBzIm)_2(acr)_4]$ (**45**) and $[Cu_2(5,6\text{-}Me_2BzIm)_2(acr)_4]$ (**47**), and the low cytotoxicity of $[Cu(2\text{-}MeBzIm)_2(acr)_2]$ (**44**) and $[Cu(5,6\text{-}Me_2BzIm)_2(acr)_2(H_2O)] \cdot 2H_2O$ (**48**);
- Studies regarding the antiinflammatory activity evidenced compunds (**16**) and (**48**) as being as active as diclofenac drug (which was the reference drug in these studies), presenting a very good antiinflammatory effect.

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List of published articles (with PhD thesis subject):

1. Ioana Dorina Vlaicu, Rodica Olar, Dana Marinescu, Veronica Lazar, Mihaela Badea, Physico-chemical and thermal characterization of new Co(II) complexes with pyrazole derivatives, Journal of Thermal Analysis and Calorimetry, 2013, 113:1337-1343. **[FI = 1,982]**

2. Ioana Dorina Vlaicu, Madalina Constand, Rodica Olar, Dana Marinescu, Maria Nicoleta Grecu, Veronica Lazar, Mariana Carmen Chifiriuc, Mihaela Badea, Thermal stability of new biologic active copper(II) complexes with 5,6-dimethylbenzimidazole, Journal of Thermal Analysis and Calorimetry, 2013, 113:1369-1377. **[FI = 1,982]**

3. Mihaela Badea, Ioana Dorina Vlaicu, Rodica Olar, Madalina Constand, Coralia Bleotu, Mariana Carmen Chifiriuc, Luminita Marutescu, Veronica Lazar, Maria Nicoleta Grecu, Dana Marinescu, Thermal behaviour and characterisation of new biologically-active Cu(II) complexes with benzimidazole as main ligand, Journal of Thermal Analysis and Calorimetry, 2014 –DOI: 10.1007/s10973-014-3745-z - acceptată pentru publicare. **[FI = 1,982]**

Other publications:

1. S.V. Nistor, D. Ghica, M. Stefan, I. Vlaicu, J.N. Barascu, C. Bartha, Magnetic defects in crystalline $Zn(OH)_2$ and nanocrystalline ZnO resulting from its thermal decomposition, Journal of Alloys and Compounds, 2013, 548:222-227.

2. Rodica Olar, Gina Vasile Scaeteanu, Ioana Dorina Vlaicu, Luminita Marutescu, Mihaela Badea, Synthesis, physico-chemical characterization and thermal behaviour of new complexes with N_4O_2 donor set, Journal of Thermal Analysis and Calorimetry, 2014 – In press.

Communications (poster or oral presentations)
1. New biologic active Cu(II) complexes with benzimidazole as one of the ligands: Synthesis, characterization and thermal behavior/ Ioana Dorina Vlaicu, Rodica Olar, Dana Marinescu, Mihaela Badea, Mariana Carmen Chifiriuc/ 2nd Central and Eastern European Conference on Thermal Analysis and Calorimetry, CEEC-TAC2 2013, Vilnius (Lituania), 27-30 August (2013) – prezentare tip poster/359.

2. New cobalt(II) complexes with mixed ligands as antimicrobials/ Ioana Dorina Vlaicu, Mihaela Badea, Rodica Olar, Dana Marinescu, Mariana Carmen Chifiriuc, Luminita Marutescu, Veronica Lazar/ 2nd Central and Eastern European Conference on Thermal Analysis and Calorimetry, CEEC-TAC2 2013, Vilnius (Lituania), 27-30 August (2013) – prezentare tip poster/360.

3. Three new iron(III) complexes with Schiff base type ligands : synthesis, spectral and thermal characterization/ Ioana Dorina Vlaicu, Mihaela Badea, Rodica Olar, Florentina Patrascu, Dana Marinescu/ Work-shop-ul « New trends in materials science »/Bucuresti (Romania) 28-31 Martie 2012/ prezentare tip poster.

4. Synthesis, spectral, thermal and biological characterisation of new iron complexes with Schiff base ligands/ Ioana Dorina Vlaicu, Mihaela Badea, Florentina Patrascu, Rodica Olar, Dana Marinescu/ 1st Central and Eastern European Conference on Thermal Analysis and Calorimetry, CEEC-TAC1 2013, Craiova (Romania), 7-10 Septembrie (2011) – prezentare tip poster/415.

5. New iron(III) complexes with pentadentate schiff base ligands: synthesis, spectral, thermal and biological characterization/ I.D. Vlaicu, M. Badea, R. Olar, D. Marinescu/ The 7th international conference on advanced materials, ROCAM 2012, Braşov (România) 28-31 August 2012 – prezentare orală/ 46.

6. Thermal stability of new biologic active copper (II) complexes with 5,6-dimethylbenzimidazole, Ioana D. Vlaicu, Madalina Constand, Rodica Olar, Dana Marinescu, Maria N. Grecu, Veronica Lazar, Mariana C. Chifiriuc, Mihaela Badea, *ICTAC15* (15thInternational Congress on Thermal Analysis and Calorimetry), 20-24 August 2012, Osaka, Japonia, Stick of Abstracts IC-IM-PS-21 – prezentare tip poster.

7. Physico-chemical and thermal characterization of new Co(II) complexes with pyrazole derivatives , Ioana D. Vlaicu, Rodica Olar, Dana Marinescu, Veronica Lazar, Mihaela Badea , ICTAC15 (15th International Congress on Thermal Analysis and Calorimetry), 20-24 August 2012, Osaka, Japonia, Stick of Abstracts IC-IM-PS-22 – prezentare tip poster.