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Structural studies of metal complexes with amino acids and biomarkers for use in diagnostic

Doctoral Thesis Summary

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Table of contests

Keywords: amino acids, biomarkers, metal compounds, GC-MS, UV-VIS

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INTRODUCTION

In recent years the importance of metal ions for smooth operation of plant and animal organisms was evidenced by the publication of numerous papers in the field of biophysics and biochemistry. Latest researches in these areas focus on the synthesis and characterization of biological compounds containing metal ions, due to their applicability in pharmacy, medicine, agronomy and nutrition. In recent years, an increased focus on studies carried out on the complexation of transition metal ions with different biomolecules.

Amino acid complexes with metals are similar in structure to the natural ones present in the body and release trace elements is exactly the cell or tissue that needs them.

In literature are reported various applications of metal complexes with amino acids

- in medicine as antibacterial activity (complexes with Cu-alanine, arginine, histidine, lysine) on bacteria like Staphylococcus aureus, Streptococcus pyogenes and Escherichia coli in regulating gastric (Cu-tryptophan, phenylalanine) as antitumour (Cu-leucine, tyrosine, histidine
- in pharmacy that bacteriostatic drugs (Co-cysteine, methionine) as inhibitors of HSV-1 virus (Co-lysine, arginine, histidine, Co-peptide), (as insulin-mimetic Zn-aspartic acid, proline, threonine, valine) as vasoconstrictors (Zn-L-histidine) used in diseases of deficiency (Fe-ac.aspartic, serine) as drugs used in metal poisoning;
- in nutrition that pig feed supplements (Zn-methionine) and restorative iron supplements for human (Fe-glycine), etc..

This paper consists in four chapters as follows: in the first chapter theoretical aspects of amino acids, the biological role of transition metals in the body, and theoretical aspects related to biomarkers.

The second chapter describes the experimental methods structural analysis.

The third chapter provides information on the samples studied, the experimental methods used and the results obtained.

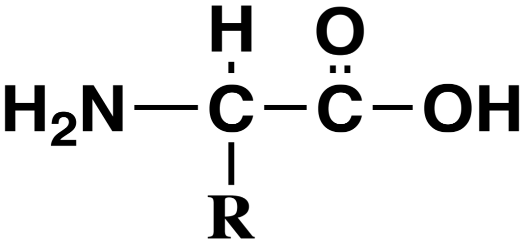
This work ends with conclusions wich result from this study and the references.

CHAPTER I

1. Amino acids

Structure, classification and biochemical role

Amino acids are biochemical compounds with mixed operation as containing a carboxyl functional group (-COOH) and amine functional group (-NH2), both grafted to the same carbon atom in α position (or C2) [1].



$H_2N-CH(R)-C(=O)-OH$

The basic structure of an amino acid

Depending on the structural features of radical R, amino acids are classified as follows:

1. Cyclic amino acids

a. Monoaminomonocarboxilici amino acids with aliphatic radical: glycine / glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile)

b. Monoaminomonocarboxilici amino acids with a hydroxyl group (hidroxiaminoacizi): serine (Ser), threonine (Thr);

c . monoaminomonocarboxilici sulfur amino acids: cysteine (Cys), cystine (Cys-Cys), methionine (Met);

d . Amino monoaminodicarboxilici: aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamine (GLN);

e. Amino diaminomonocarboxilici: lysine (Lys), hidroxilizina (Lys-OH), arginine (Arg).

2. Cyclic amino acids

a. Amino homeociclici (the aromatic nucleus) phenylalanine (Phe), tyrosine (Tyr);

b. Amino heterocyclic: histidine (His), tryptophan (Trp), proline (Pro), hydroxyproline (Pro-OH)

Depending on the character you show him side chains (R) amino acids are classified as follows:

a) apolar chain amino acid - neutral: glycine, alanine, leucine, isoleucine, methionine, phenylalanine, tryptophan;

b) polar chain amino acids: serine, threonine, cysteine, tyrosine, asparagine, glutamine;

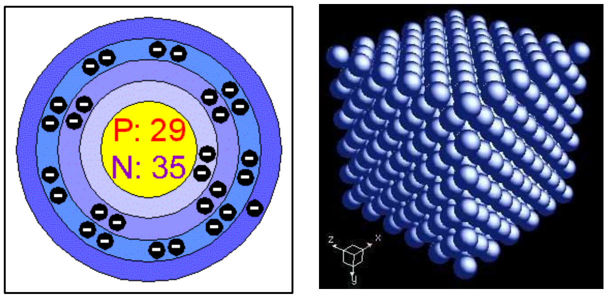
2. *The biological role of transition metals in the body (physical and chemical properties of metal complexes of biomedical interest)*

Biological activity of metal

Of all known chemical elements, nature has selected some 52 contained in living matter. In any living organism are constantly 40-43 of elements (called bioelements), of which 25 are essential for the structure and functionality. These elements fall among the most commonly found in the earth's crust, which demonstrates that natural selection has eliminated the dependence of living organisms items less accessible. Six of the 25 essential elements (C, N, H, O, P, S) is based on building living organic matter (protein, glycogen, starch, lipids and nucleic acids) [2].

1.1.1 Cooper

Copper is an essential element for living beings, with an important role in hematopoiesis and porphyrin synthesis in numerous metabolic processes and enters also into the composition of cuproenzime [3]



Electronic configuration of copper ion

The biological role of copper

Copper plays an important role in bone formation in skin pigment synthesis, eyes, hair, wound healing, participates in the synthesis of red blood cells, is important for psycho-emotional balance and influence the function of glands with internal secretion.

The body that represents more accurately the concentration of copper in the body is the liver; as a result, some authors give greater importance than other methods dosage hepatitis to assess the state of deficiency, or toxicity sufficient. Stored mainly in parenchymal cells, copper may occur in greater quantity and Kupffer cells in case of poisoning [4].

Copper complexes with amino acids

In biological systems, copper is found in the form of Cu (II), rarely Cu (I) in deoxihemocianine, and rarely Cu (III).

Function of copper in biological systems can be found in redox reactions associated with reducing

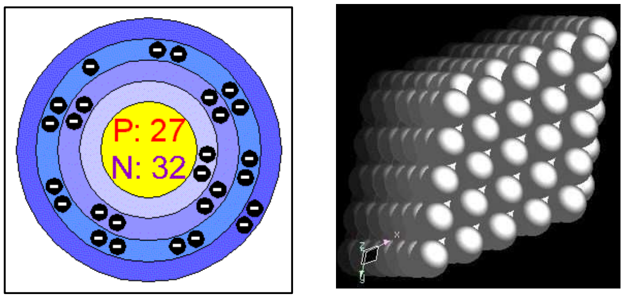
O_2 to water, O_2 transfer to the substrate, respectively monooxigen insert - oxygenase to transform phenol to diphenols and insertion dioxigen - dioxygenase, the transformation of catechol to a quinone.

Copper ion complexation with various α-amino acids is similar, except that histidine formation of connections to the nitrogen atoms and oxygen atoms aminic group of carboxylate moiety, respectively glicin-NNOO coordination type [5].

1.1.2 Cobalt

Cobalt metal is relatively less common in nature plays a very important technical importance is due to its value as a component of hard alloys (cermets) and steliţilor type casting alloys and special alloys with specific properties, magnetic, refractory and antacids [6]

Cobalt is a transition metal of Group IB, is the 27th element of the periodic table, atomic mass 58.933 amu, Density: 8.90 g/cm3 Melting point: 1495 0C 29270C boiling point has electronic configuration: [ar] 4s2 3D7.



Electronic configuration of cobalt ion

The biological role of cobalt

Cobalt is an essential trace mineral whose biological activity is manifested through its role in the series of coenzymes and vitamin B12. Vitamin B12 is important in the hematopoetic indispensable in the synthesis of hemoglobin, is also anemic and antipernicios factor.

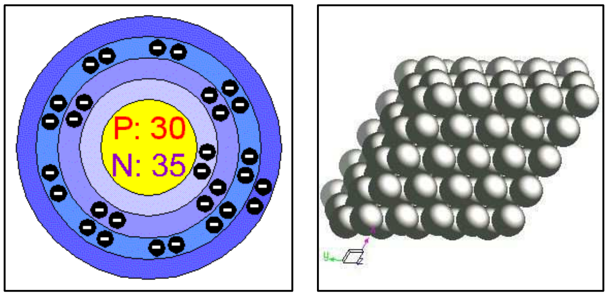
Cobalt absence of living organisms might result in severe nervous system dysfunction, anemia and unnatural developments in cell growth. To treat these symptoms, it takes the whole molecule and not just the Cobalt B12.

Cobalt complexes with amino

Recent studies on the formation of complexes of cobalt (II) with different tioaminoacids such as cysteine and methionine, due to their bacteriostatic action [7]. Also, the literature presents a series of studies on the action of divalent cobalt complexes with lysine, arginine, histidine and serine on HSV-1 virus [8]. Complexes with arginine and histidine had no effect on virus replication but inhibits virus replication complex with lysine and serine at the very best inhibitory effect (90%).

1.1.3 Zinc

Zinc is a transition metal, is the 30th element of the periodic table, atomic mass 65.39 amu, melting point: 419.58 0C, 9070C boiling point has electronic configuration: [He] 4s2 3d10 and crystalline structure is hexagonal [9].



Electronic configuration of zinc ion

The biological role of zinc

Zinc is a trace of virtues (properties) and antioxidant as an active element in the constitution fall about 60 enzymes, has an important role in: maintaining vision, protein metabolism, activity of the pancreas and sexual organs, stimulating the healing process of burns and wounds, stimulation of assimilation of vitamins, training leukocytes, immune activation, favoring nucleic acid synthesis and repair processes etc.

Zinc can be considered one of the most important trace elements due to its involvement in the construction and manufacturing cellular mechanisms. The daily range, 5-10 mg is recommended in children, in adults 12-15 mg during pregnancy is recommended to increase the intake of 20-40 mg zinc and 50 mg even if breastfeeding [10].

Zinc complexes with amino

Nutritional zinc deficiency in developing countries determines increased susceptibility to many pathogens such as fungi, viruses, bacteria [10].

Study and characterization of the interaction between tryptophan, fenilalanin as ligands and zinc ferrocyanide, cobalt, copper and nickel [10] have demonstrated that both amino acids have affinity front of ferrocyanide at pH = 7, the interaction puternică settling between Zn (II) and tryptophan.

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3. Biomarkers

Biomarker is defined as an indicator of normal biological processes, those pathological or pharmacological response to a therapeutic intervention can be assessed / measured objectively.

Biomarker may be a component of biological products (blood, urine, tissue) or can be obtained by recording (ECG) or imaging (echocardiography, CT, etc.). Ideal would be to determine genetic markers that can be represented by: gene itself, messenger RNA (mRNA) or protein encoded by mRNA [11].

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CHAPTER III. Structural studies, results

A. Spectroscopic studies investigations of some transitional metals complexes with methionine as ligand

Minerals such as zinc, copper, iron and others can chemically bond to amino acids resulting chelates. Amino acids are ideal chelators or ligands from both chemical [1] and nutritional [2] points of view. Metal amino acid chelates resemble these compounds which allow the minerals to be carried in with the amino acids during absorption. Finally, the amino acids, once released from the metal, can be used to build proteins or provide energy.

Elemental analysis

The elemental analysis results for the synthesized complexes confirm the 1:2 ratio metal/ methionine. The elemental analysis data of the metal-methionine complexes are pointed up in Table 1.

Table 1. Elemental analysis results the complexes

Symbolic formula	**Molecular weight**	**%C**		**%H**		**%N**		**%Metal**	
		Calc.	**Meas.**	**Calc.**	**Meas.**	**Calc.**	**Meas.**	**Calc.**	**Meas.**
$[Cu(L)_2]\cdot H_2O$	357.5	33.56	31.50	5.5	5.72	7.8	7.27	17.9	17.26
$[Co(L)_2]\cdot 2H_2O$	353	33.59	30.75	5.66	6.21	7.93	7.41	18.13	17.76
$[Zn(L)_2]\cdot H_2O$	359.6	33.39	33.14	5.56	6.33	7.69	6.74	17.81	16.19

Atomic absorption spectroscopy

The atomic absorption results for the synthesized complexes are in concordance to those theoretical obtained (Table 2).

Table 2. Metal concentrations obtained by means of atomic spectroscopy

Complex	**Metal concentration from the complex (%)**	
	Calc.	**Meas.**
1	17.44	17.43
2	16.05	15.77
3	15.66	16.47

Differential Scanning Calorimetry

The ligand trace indicate an endothermic reaction at 275 °C specific to pure substances and an exothermic reaction between 310 to 360°C attributed to the decomposition and combustion of organic matter components. The thermogram of complex **1** indicates a melting process fallowed by decomposition between 230 to 270 °C and the several exothermic peaks between 270 to 420 °C are attributable to the oxidation of the decomposing product. The complete combustion took place subsequent to 500 °C. The complex **2** thermogram indicates a more complex plot with peaks owing to dehydration, decomposition, oxidation and combustion processes. Between 80 to 100 °C two successive endothermic peaks appeared and were attributed to dehydration processes. After 100 °C a two step decomposition processes took place between 130 to 200°C and 150 to 170°C, and an intermediary product was formed. The exothermic from 380°C and 400°C were associated to the oxidation of the decomposing products. The complete combustion took place subsequent to 480 °C. The DSC trace of complex **3** indicates an exothermic reaction between 130 to 150 °C attributed to the crystallization process. The melting point appears at lower temperature (220 °C) than the one for the ligand (275°C), which profs the complex formation. The complete combustion took place subsequent to 450 °C. The DSC trace for all samples are given in Fig. 1.

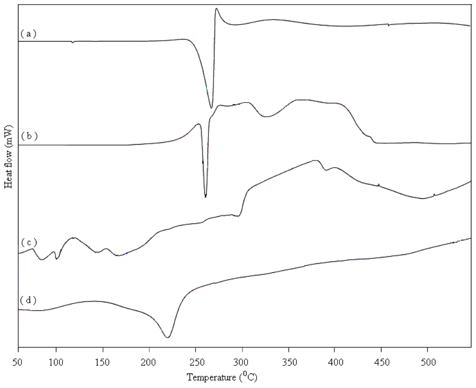

Fig 1. DSC thermograms of L (a), 1 (b), 2 (c) and 3(d)

FT-IR spectroscopy

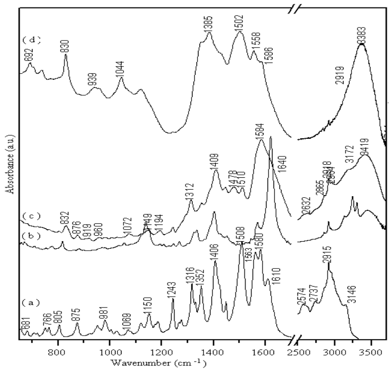

Fig. 2 FT-IR spectra of **L** (a), **1** (b), **2** (c) and **3** (d)

In the ligand spectra the ν(N-H) stretching vibration appears at 3146cm^{-1} and is shifted at 3229 cm^{-1}, 3172 cm^{-1} and 3450 cm^{-1} in the Cu(II), Co(II) and Zn(II) spectra proving the involvement of the –NH_2- group in the complex formation [3, 4, 5]. The CH_2-S and CH_3-S stretching vibrations appears as a sharp band at 2915 cm^{-1} in the ligand spectrum and are insignificant shifted in the complexes spectra confirming the non involvement to the coordination. The absorption band from 1610 cm^{-1} in the ligand spectrum was attributed to the ν(C=O) stretching vibration and appears to be shifted toward higher wave numbers in the complexes **1** and **2** spectra, which involves the carboxylic group in the covalent bonding to the metal ion [6]. The ν (OH) stretching vibrations do not emerge in the ligand spectrum, but they appear in the complexes spectra at values between 3383 to 3449 cm^{-1}, suggesting the presence of the crystallisation water within these complexes.

UV-VIS spectroscopy

The local symmetry around the metallic ions was determined comparing the amino acid and metallic complexes UV-VIS spectra.

The $n \rightarrow \pi^*$ characteristic band in the UV spectra assigned to the C=O bond appear at 267 nm for threonine (Fig.3.a) and is shifted toward higher wave lengths with 8 nm, 7 nm and 2 nm for **1**, **2** respectively **3** confirming the presence of the ligand in the complex and the covalent nature of the metal-ligand bond. In the visible domain (Fig.4) a d-d transition appears between at 625 nm in the copper complex spectrum assigned to the ${}^2T_{2g} \rightarrow {}^2E_g$ transition, specific for Cu (II) complexes with tetragonal distortion owing to the Jahn-Teller effect. In the visible domain, the Co-L spectrum shows a band at 512 nm attributed to the *d-d* transition of cobalt electrons. Based on the d^7 diagram in Oh field the fallowing attribution were maid: ν_1 = 8100cm^{-14} T_{2g}(F) $\rightarrow {}^4T_{1g}$(F), ν_2= 16000cm^{-1} ${}^4A_{2g}$(F) $\rightarrow {}^4T_{1g}$(F), ν_3 = 19400cm^{-1} ${}^4T_{1g}$(P) $\rightarrow {}^4T_{1g}$(F).The last band within the spectrum was assigned to the ${}^4T_{1g}$ (P)$\rightarrow$ ${}^4T_{1g}$(F) transition, expected for an octahedral symmetry of cobalt ion.

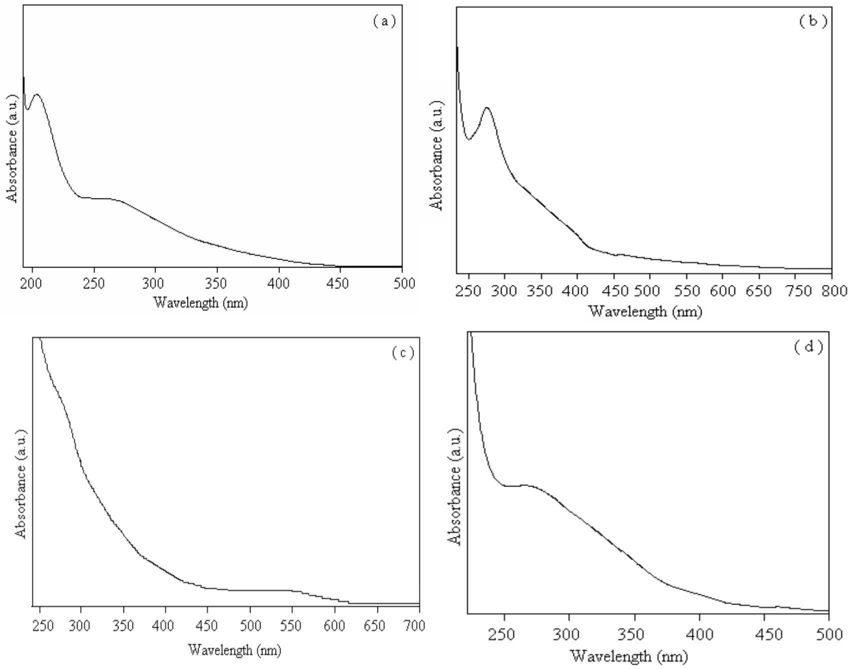

Fig. 3 UV and VIS spectra of **L** (a), **1** (b), **2** (c) and **3** (d)

EPR spectroscopy

Powder EPR spectrum at room temperature are typically for pseudotetrahedral symmetry around the copper ion with the g tensor value: g=2.094 corresponding to a CuN_2O_2

cromophore [7, 8]. The Co-L powder EPR spectrum revealed the presence of monomeric compounds, with octahedral symmetry around the cobalt ion, the g tensor value is g=2.201 [9].

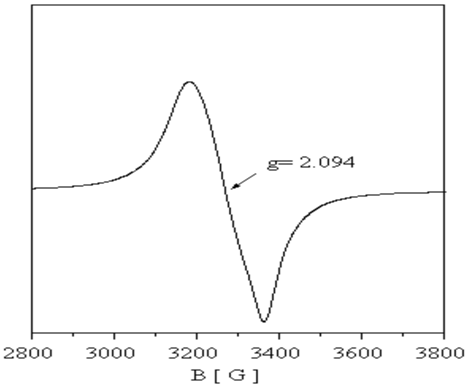

Fig. 4 Powder ESR spectrum of complex **1**

Conclusions

Three new metallic complexes with methionine as ligand were synthesized and analyzed by means of: elemental analysis, atomic absorption, IR, UV-VIS and EPR spectroscopies.

The study reason was to compare the complexation capacity of the amino acids with the copper ion in similar synthesized conditions to choose those complexes which are easy to synthesize, with high yield, to use it as a method of extraction and separation of the amino acids from mixtures.

The atomic absorption spectroscopy and elemental analysis confirms the stoichiometry of the compounds, the composition corresponded to a metal-ligand ratio of 1:2. The IR spectra show that amino acids act as bidentate ligands with the coordination involving the carbonyl oxygen and the nitrogen atom of amino group. The EPR spectra confirm the pseudotetrahedral local symmetry for copper ion and octahedral symmetry for cobalt ion.

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B. Preliminary investigation of mocs meteorite by lead isotopic ratio using quadrupole inductively coupled plasma mass spectrometry

Mocs meteorite, classified as L5-6 chondrite [1] fell as a shower of stones on 3rd February 1882, in Transylvania region over an area of several dozen squared kilometers near Mocs village (now Mociu, Cluj County, Romania, coordinates 46°48' N, 24°2' E). The number of recovered fragments was estimated at 3000, with a total weight of about 300 kg [2]. According to the Meteoritical Bulletin Database, Mocs is one of the 31 approved meteorites classified as L5-6. The exact fall location of the investigated Mocs meteorite sample (inv. no. I.34B) is Chesău village (Keszu, in the official catalogues of meteorites, e.g. [2]), Mociu Commune, Cluj County). Due to the large amount of material present in more than 30 museums worldwide [2], Mocs meteorite has been intensely studied before; however, no lead isotopes measurements have been done so far. Our experiments concerned whole rock fragments.

For this study, ^{206}Pb, ^{207}Pb and ^{208}Pb were selected for measurement. Due to isobaric interferences, the quadrupole cannot differentiate between ^{204}Pb and ^{204}Hg isotopes and mercury was found at a level of 1.047 mg/kg (other studies [3] found 1.120 mg/kg), which is impossible to ignore in our lead measurements. For terrestrial samples with known Hg isotopic ratio, mathematical correction can be performed for subtracting ^{204}Hg counts from the total counts at mass 204. Since no data about Hg isotopic ratio in Mocs meteorite are yet provided, no such mathematical correction could be applied; interferences from Hg would have caused a false signal on mass 204 that would have rendered correct ^{204}Pb determination to be impossible with current method. Isotopic ratio determination for Hg by hydride generation would be able to improve ^{204}Pb measurements.

Conclusions

An inductively plasma mass spectrometer with single detector and a quadrupole for mass discrimination was used for lead isotopic ratio measurements on Mocs meteoritical sample. The proposed method was tested using a NIST 981 standard reference material and it offered good results for lead determination. Even if the calculation of radiometric ages is not possible by using the current results, they may provide some comparative grounds for Pb isotope compositions on other chondritic meteorites. Further method improvements for including determination of ^{204}Pb isotope is currently underway.

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C. Spectroscopic studies of manganese (II), cooper (II) and palladium (II) complexes with diclofenac as ligand

Diclofenac sodium, (2-[2,6-dichlorophenilamino] phenylacetate) (L) and its complexes with transition metal are potent non-steroidal anti-inflammatory drugs, that have been used to alleviate the pain and edema associated with rheumatoid arthritis, osteoarthritis, spondylitis, and many other inflammatory conditions [1, 2]. The exact mechanism of action is not entirely known, but it is thought that the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX) and it appears to inhibit DNA synthesis.[5]Inhibition of COX also decreases prostaglandins in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This is also the main side effect of diclofenac. Diclofenac has a low to moderate preference to block the COX2-isoenzyme (approximately 10-fold) and is said to have therefore a somewhat lower incidence of gastrointestinal complaints than noted with indomethacin and aspirin [3, 4].

Elemental analysis

Complexes were synthesized in good yields (62% (**1**), 68 % (**2**) and 65 % (**3**) and the elemental analyses results (Table 1) confirm their stoichiometry.

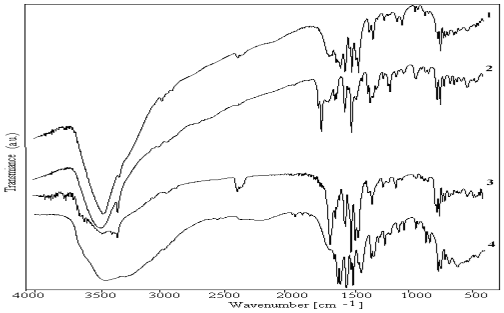
Table 1 Analytical data of the synthesized complexes

	Colour	**Found (Calcd.) (%)**				
		C	**H**	**N**	**Cl**	**M**
1	pale pink	51.0 (50.7)	3.5 (3.3)	4.0 (4.2)	21.1 (21.4)	8.1 (8.2)
2	brown	46.2 (45.9)	3.5 (3.3)	3.7 (3.8)	19.7 (19.3)	14.7 (14.5)
3	green	49.1 (48.7)	3.8 (3.5)	3.7 (4.0)	20.4 (20.6)	9.5 (9.2)

FT-IR spectroscopy

In the $\nu(OH)_{water}$ region spectra of the (**1**) and (**3**) show a broad absorption at ~ 3500 cm^{-1} attributed to the presence of coordinated water. The strong band at 3388 cm^{-1} which appear in diclofenac, is assigned to the ν(NH) stretching motion and the broad band at 3260 cm^{-1} is taken to represent the ν(NO) mode, due to intramolecular hydrogen bonding [5-8]. The complexes (**1)** and (**3)** exhibit bands at 3563 and 3568 respectively, attributed to the presence of coordinated water and **L,** complexes **(2)** and **(3)** exhibit broad bands at 3456, 3470 and 3446 respectively attributed to the lattice water

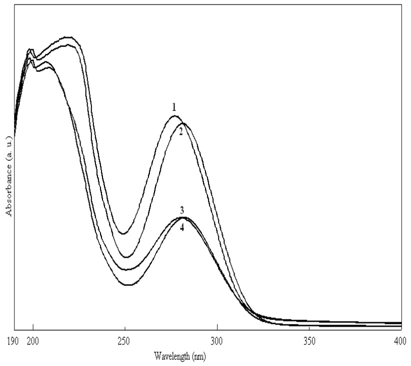
The absence of large systematic shifts of the ν(NH) and δ(NH) bands in the spectra of these complexes compared with those of the ligand indicates that there is not interaction between the NH group and the metal ions.



FT-IR spectra of **1-** $[MnL_2(H_2O)]$, **2 -** $[PdL_2]\cdot 2H_2O$, **3 -** $[CuL_2(H_2O)]_2\cdot 2H_2O$ and **4**-Natrium-diclofenac in KBr pellets

Electronic Spectroscopy

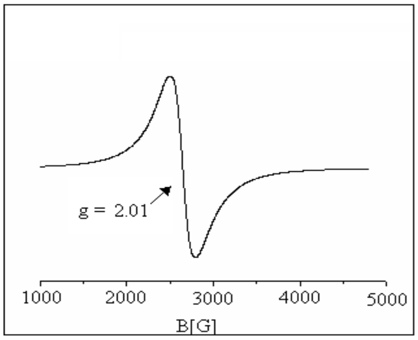
The electronic spectrum of the ligand presents three absorption bands at ν_1= 199 nm, ν_2= 209.5 nm, and ν_3= 282 nm, respectively. The ν_1 band is assigned to $\pi \rightarrow \pi^*$ transitions in conjugate organic systems, the ν_2 band is characteristic for $\pi \rightarrow \pi^*$ transitions in carboxylic group and the ν_3 band caused by the $n \rightarrow \pi^*$ transitions in aromatic system. The electronic UV absorption spectra of the metal complexes are similar. The presence of different cations leads to a shift of the ν_2 bond towards higher (complex **3**) or lower (complexes **1** and **2**) energies in these spectra. These shifts might be correlated with involvement of both oxygen atoms from the carboxylic groups of the ligand in the metal coordination, which caused a strengthening of the C-O bonds involved in the charge transfer processes for complex **3** and weakening of these bonds in the **1** and **2** complexes [9, 10].



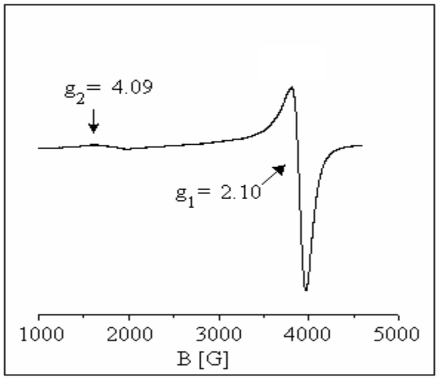
UV Spectra of the **1**-$[PdL_2]\cdot 2H_2O$, **2** - $[MnL_2(H_2O)]$**,** **3** - $[CuL_2(H_2O)]2\cdot 2H_2O$ and **4** - $NaL\cdot 4H_2O$, in methanol ($c=10^{-5}$ M).

RES spectroscopy

The polycrystalline powder ESR spectrum of $[MnL_2(H_2O)]$ complex, at room temperature is characterized by quasi-isotropic g tensor with the principal value (g=2.01) close to the spin only value [11]. The signal at g ≈ 4 in the powder ESR spectrum of the Cu(II) complex suggests the presence of the dimeric species due to the dipol-dipol interaction between the metallic ions [12].



Powder ESR spctrum of complex (**1**)



Powder ESR spctrum of complex (**3**)

Conclusions

Metal (II) complexes of diclofenac with interesting anti-inflammatory profiles have been prepared and studied by infrared, electronic and ESR spectroscopic methods.

The square pyramid geometry with a water oxygen occupying both apical positions in copper complex, one apical position in manganese complex and square plan geometry in palladium complex results from analytical and spectroscopic data.

The spectroscopic measurements results confirmed the metal-ligand bonds, the square-pyramidal symmetry of complexes (**1**) and (**3**) and the tetrahedral symmetry of complex (**2**).

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D. Diagnosis and Control by Using Biomarkers

New biomarkers are needed in the biomedical research as measurable indicators of normal and pathological processes, with the target of rapid and sure diagnosis, by using advanced techniques.

Non-invasive and semi-invasive methods were developed for the quantitative determination of the biomarkers: valine, leucine, proline, phenylalanine and tyrosine for PKU and MSUD inborn error metabolic diseases. Gas chromatography - mass spectrometry (GC-MS) demonstrates to be an indispensable method for diagnosing inborn error of metabolism and is widely recognized for its effectiveness in related fields [1, 2].

The effects of liver disease on some pharmacokinetic parameters of caffeine as clearance and half-life time were also studied by (GC/MS) [3, 5].

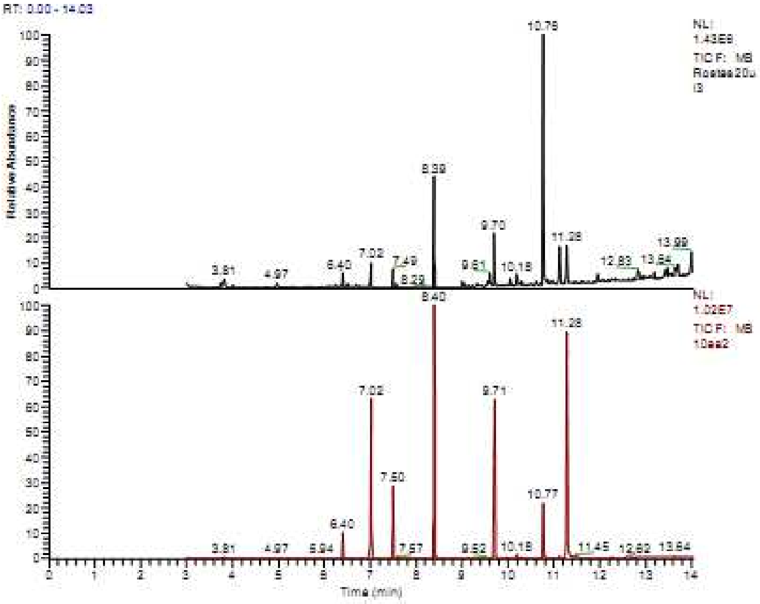

Fig. 1 shows the chromatogram of separation of the five amino acid by using the minim invasive method, from 20 µl blood. The analysis being performed in the SIM mode by using the ions selected from the trifluoroacetyl butyl ester derivatives mass pectra:, m/z 168 for valine, m/z 182 for leucine, m/z 183 for 15 used as internal standard m/z 166 for proline, m/z 91 and 148 for phenylalanine and m/z 203, 260, 316 for tyrosine.25µg.ml-1 of the internal standard was added at each sample.

<u>Conclusions</u>

GC-MS is a suitable method for PKU diagnosis in neona tal blood samples, from Phe /Tyr ratio and MSUD from three amino acid values. The measurements performed on amino acids from dried blood spots showed good precision and accuracy.

In cirrhotic patients, the caffeine test showed the elimination half-life (t1/2) of caffeine significantly longer and clearance substantially reduced than in control. Caffeine pharmacokinetic parameters can be estimated using two point blood sampling procedure by GC-MS determination, following a single load.

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CHAPTER IV. Conclusions

Three new metallic complexes with methionine as ligand were synthesized and analyzed by means of: elemental analysis, atomic absorption, IR, UV-VIS and EPR spectroscopies.

The study reason was to compare the complexation capacity of the amino acids with the copper ion in similar synthesized conditions to choose those complexes which are easy to synthesize, with high yield, to use it as a method of extraction and separation of the amino acids from mixtures.

The atomic absorption spectroscopy and elemental analysis confirms the stoichiometry of the compounds, the composition corresponded to a metal-ligand ratio of 1:2. The IR spectra show that amino acids act as bidentate ligands with the coordination involving the carbonyl oxygen and the nitrogen atom of amino group. The EPR spectra confirm the pseudotetrahedral local symmetry for copper ion and octahedral symmetry for cobalt ion.

An inductively plasma mass spectrometer with single detector and a quadrupole for mass discrimination was used for lead isotopic ratio measurements on Mocs meteoritical sample. The proposed method was tested using a NIST 981 standard reference material and it offered good results for lead determination. Even if the calculation of radiometric ages is not possible by using the current results, they may provide some comparative grounds for Pb isotope compositions on other chondritic meteorites. Further method improvements for including determination of ^{204}Pb isotope is currently underway.

Metal (II) complexes of diclofenac with interesting anti-inflammatory profiles have been prepared and studied by infrared, electronic and ESR spectroscopic methods.

The square pyramid geometry with a water oxygen occupying both apical positions in copper complex, one apical position in manganese complex and square plan geometry in palladium complex results from analytical and spectroscopic data.

The spectroscopic measurements results confirmed the metal-ligand bonds, the square-pyramidal symmetry of complexes (**1**) and (**3**) and the tetrahedral symmetry of complex (**2**).

GC-MS is a suitable method for PKU diagnosis in neona tal blood samples, from Phe /Tyr ratio and MSUD from three amino acid values. The measurements performed on amino acids from dried blood spots showed good precision and accuracy. In cirrhotic patients, the caffeine test showed the elimination half-life (t1/2) of caffeine significantly longer and clearance substantially reduced than in control. Caffeine pharmacokinetic parameters can be estimated using two point blood sampling procedure by GC-MS determination, following a single load.