Synthesis and Characterization of Some Cobalt (II) Complexes with Amino Acids Having Biological Activities

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*Cobalt-amino acids complexes in aqueous solution: $[Co-(L_1H_2O)_2]\times H_2O$ **(1)**, (L_1=lysine), $[Co-(L_2H_2O)_2]\times H_2O$ **(2)**, (L_2=leucine) and $[Co(L_3H_2O)_2]\times 2H_2O$ **(3)**, (L_3=methionine) were synthesized and characterized by means of elemental, thermal and IR, UV-VIS, and EPR spectroscopic investigations, and biological measurements. The IR spectra show that amino acids act as bidentate ligands with coordination involving the carboxylic oxygen and the nitrogen atom of the amino group. The ν(C=O), ν(N-H), and δ(N-H) vibrations are shifted toward higher frequencies for complexes comparable with ligands. Visible electronic and powder EPR spectres at room temperature are typical for monomeric species with octahedric local symmetry around the metal ion. The biological testing of the synthesized complexes was done to see citotoxic activity that might show on cell cultures of Sacharomices cerevisiae and Candida albicans.*

Keywords: amino acids, Co(II) complexes, FT-IR/UV-VIS/EPR spectroscopy, biological testing

In recent years transition metals amino acids complexes have received much attention because they proved to be useful antibacterial agents applied against *Staphylococcus aureus, Escherichia coli*, nutritive supplies for humans and animals, etc [1].

Twenty natural amino acids comprise the building blocks of proteins, which are chemical species indispensable to perform a large number of biological functions, [2]. From these twenty amino acids, eight are essential and cannot be produced by the human body. Lysine, leucine and methionine are three of these essential amino acids.

Complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes, such as oxygen conveyer, electron transfer and oxidation. In these processes, the enzymatic active site, which is very specific, forms complexes with divalent metal ions [3].

L-Lysine is a necessary building block for all protein in the body. L-Lysine plays a major role in calcium absorption, building muscle protein, recovering from surgery or sports injuries and the body's production of hormones, enzymes and antibodies.

It has been suggested that lysine may be beneficial for those with herpes simplex infections [4].

Leucine helps with the regulation of blood-sugar levels, the growth and recover of muscle tissue, growth hormone production, wound healing as well as energy regulation. It can assist to prevent the breakdown of muscle proteins that sometimes occurs after trauma or severe stress. It may also be beneficial for individuals with phenylketonuria - a condition in which the body cannot metabolize the amino acid phenylalanine [5].

Methionine is one of the amino acids containing sulfur, it helps to prevent disorders of the hair, skin and nails, in lowering the cholesterol levels by increasing the liver's production of lecithin and reduces fat build-up in the liver and body [6].

Experimental part

Methods

The Vario El device allows the quantitative determination of the carbon, nitrogen, hydrogen, sulfur, and oxygen in various operating modes. Cobalt dosing was done by a spectrophotometric method usually used to determinate Co in Vitamin B_{12}.

The thermogravimetric analysis (TG) were carried out using a Q-1500 D derivatograph, in the temperature range of 20 to 800 ^{0}C, at a heating rate of 10^{0}C min^{-1}. The analyses were carried out over samples varying from 100 to 300 mg.

FT-IR spectra were taken with a Perkin-Elmer FT-IR 1730 spectrophotometer on KBr solid samples in 4000 - 400 cm^{-1} range.

UV and visible electronic spectra were recorded in the λ=190-800 nm range in aqueous and DMFA solutions using a standard Jasco V-530 spectrophotometer.

Powder EPR measurements were performed at room temperature at 9.4 GHz (X band) using a standard JEOL-JES-3B equipment.

For *in vitro* biological measurements were used cells culture of *Saccharomices cerevisae* and *Candida albicans*.

The resistance of *Saccharomices cerevisae* mutants to high concentration of Co^{2+} was studied in [20].

Beer yeast grown on complete medium contains: 2% glucose, 1% yeast-extract, 0.5% peptone at 28-31^{o}C. Yeast where co-cultivated in the presence of three different amino acid-metal complexes, at three concentrations: 10^{-3}, 10^{-4} and 10^{-5} M.

For the other cell line it was used *Candida albicans* (CA) selected strain (ATCC10231, CULTILOOPS), standardised cell culture medium, Hexoral as positive control (antifungal product on the market at 1:10 dilution).

CA is co-cultivated in the presence of cobalt-lysine and cobalt leucine complexes 10^{-3}, 2 x 10^{-3} and 3 x 10^{-3}M, (10 µL/mL complex, free amino acid or metal in the cell culture medium).

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Proliferation rate was measured densitometrically at 3, 6 and 24 h, using a calibration curve for yeast cell number.

LDH activity was determined spectrophotometrically at 340nm using the reaction: Pyruvate + NADH + $H^+ \rightarrow$ L-lactate + NAD^+. Speed of NADH oxidation is directly proportional with enzymatic activity of the LDH.

For lipid peroxidation it was used 1 ml yeast homogenate mixed with 2 mL working solution containing 15% (w/v) thiobarbituric acid, 0.25 N HCl and heated for 15 min in boiling water. After cooling, the precipitate was removed by centrifugation at 1000 *g* for 10 min. Absorbance was determined at 535 nm.

Synthesis of cobalt amino acids complexes

The purpose of the study was to obtain neutral complexes of $[Co(LH_2O)_2] \times nH_2O$ type at *p*H=8-10, in the presence of a strong basis (NaOH) to obtain the ionization conditions of the amino acid (lysine, leucine and methionine).

The complexes were prepared following the procedure described in the literature [7]: 2 mmols of $\mathbf{L_1}$ (0.292 g), $\mathbf{L_2}$ (0.262 g) or $\mathbf{L_3}$ (0.286 g) were dissolved in 20 mL distilled water and for deprotonation of the amino acids 0.33 mL 30% NaOH was added. Then 1 mmol of the metal salt (0.237 g of $CoCl_2 x6H_2O$) was dissolved in 2 ml of distilled water, and was added to the deprotonated amino acid solution under stirring for several minutes. The precipitate was filtered off, washed with water several times, and dried in air.

For all amino acids precipitation was instantaneous, and were pinkish-blue for $[Co(L_1H_2O)_2] . H_2O$ (η=57.8%), pink for $[Co(L_2H_2O)_2]x\ H_2O$ (η=69.6%) and for $[Co(L_3H_2O)_2] \times 2H_2O$ (η=72.7%).

Results and discussions

Elemental analysis

Measurements of the carbon, nitrogen, hydrogen and sulfur, and spectrophotometric measurements of the cobalt confirm the 1:2 cobalt ion to ligand composition for the synthesized complexes.

Data of the elemental analysis results for cobalt amino acids complexes are illustrated in table 1.

Thermogravimetric differential analysis

The weight loss profiles indicate the amount of weight loss depending the degradation temperature ranges [8].

Thermal stability domains, melting points, decomposition phenomena and their assignments for the cobalt amino acids complexes are summarized in table 2.

Table 1
ELEMENTAL ANALYSIS DATA FOR COBALT(II) AMINO ACIDS COMPLEXES

Compound	Formula Weight	Colour	Yield %	Melting Point (^{0}C)	% Found (Calc.)				
					C	H	N	S	Co
$[Co(L_1H_2O)_2].H_2O$	403.32	pinkish -blue	57.80	200	35.73 (35.84)	7.99 (8.16)	13.88 (14.00)	-	14.61 (14.81)
$[Co(L_2H_2O)_2].H_2O$	373.30	pink	69.60	185	38.60 (38.75)	8.09 (8.14)	7.50 (7.64)	-	15.78 (15.96)
$[Co(L_3H_2O)_2].2H_2O$	427.40	pink	72.70	180	28.02 (28.10)	6.42 (6.60)	6.45 (6.57)	14.88 (15.00)	13.60 (13.78)

Table 2
THERMAL DATA OF THE COBALT (II) - AMINO ACIDS COMPLEXES

Compound	Temp. range (^{0}C)	DTG peak (^{0}C)		TG weight loss (%)		Assignment
		Endo	Exo	Calc.	Found	
$[Co(L_1H_2O)_2].H_2O$	20-200	110	-	4.46	4.52	One mole of crystal water
		-	-	-	-	One mole of coordination water
		145	-	4.46	4.47	
		-	-	-	-	One mole of coordination water
		165	-	4.46	4.51	
		-	-	-	-	Melting point
		200	-	-	-	Organic rest 2 $C_4H_{10}N$
	200-350	-	315	35.76	35.82	2 $C_2H_3NO_2$
	350-500	-	465	36.22	35.98	CoO residue
		-	-	18.57	18.80	
$[Co(L_2H_2O)_2].H_2O$	20-200	115	-	4.82	4.94	One mole of crystal water
		-	-			One mole of coordination water
		140	-	4.82	4.84	
		-	-	-	-	One mole of coordination water
		160	-	4.82	4.86	
		-	-	-	-	Melting point
		185	-	-	-	Organic rest 2 C_4H_9
	200-350	-	320	30.59	30.65	2 $C_2H_3NO_2$
	350-500	-	470	39.13	38.95	CoO residue
		-	-	20.07	20.15	
$[Co(L_3H_2O)_2].2H_2O$	20-200	105	-	8.43	8.46	Two mole of crystal water
		-	-	-	-	One mole of coordination water
		150	-	4.21	4.26	
		-	-	-	-	One mole of coordination water
		170	-	4.21	4.20	
		-	-	-	-	Melting point
		180	-	-	-	Organic rest 2 C_3H_7S
	200-350	-	320	35.16	35.22	2 $C_2H_3NO_2$
	350-500	-	475	34.18	34.03	CoO residue
		-	-	17.53	17.75	

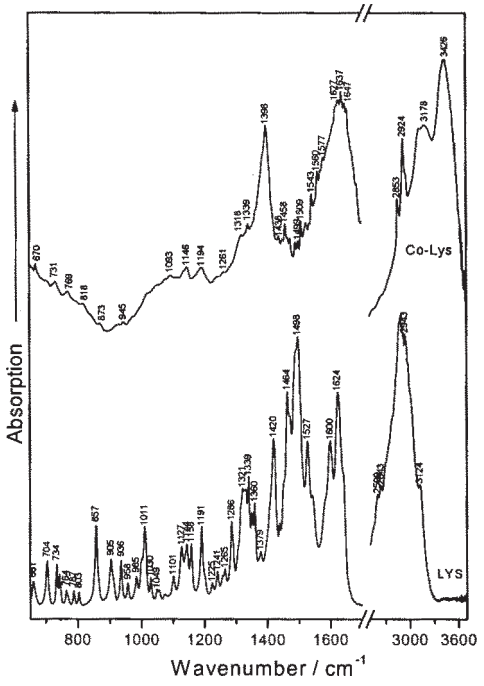

Fig. 1. IR Spectra of $\mathbf{L_1}$ and **1**

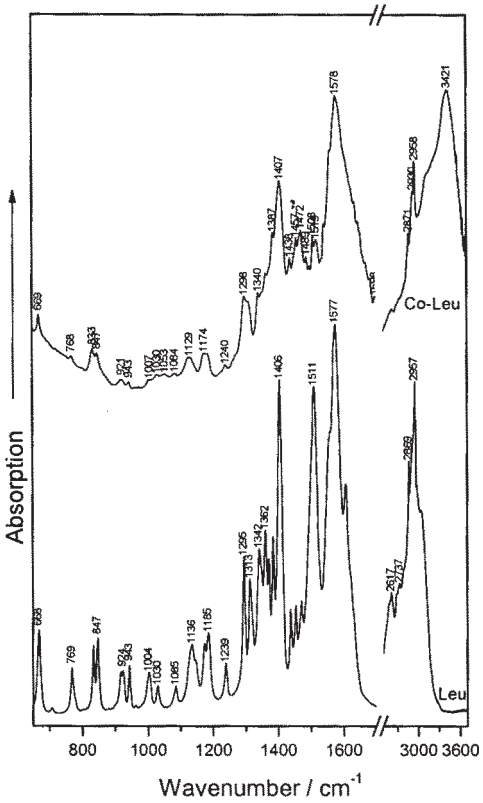

Fig. 2. IR Spectra of $\mathbf{L_2}$ and **2**

The analysis of thermal curves of the complexes clearly indicates that the weight loss between 20-110 ^{0}C corresponds to one crystal water molecule for first two complexes. [9]. The endothermic peaks between 140 and 170 ^{0}C correspond to the loss of coordinated water molecules in two steps. The sharp endothermic peak occuring between 180-200 ^{0}C may be due to melting of the complexes without weight loss.

In the temperature range 200-500 ^{0}C the exotermic peaks in the DTA curves indicated the succesive two decompositions steps. The exothermic peak at ~320 ^{0}C may be due to loss of two molecules of organic radicals

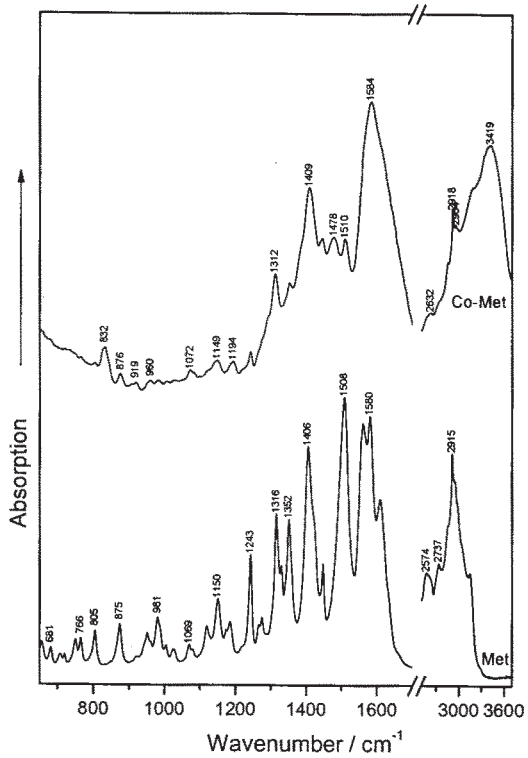

Fig. 3. IR Spectra of $\mathbf{L_3}$ and **3**

(C_4H_{10}-izobutil, C_4H_8-NH_2-amino butyl and C_3H_7S- metyl-thio-ethyl) from the ligand. The second exothermic peak at ~ 470 ^{0}C, correspond to the pyrolysis of the amino acid rest. The complexes are stable up to ca. 320 beyond which they start decomposing. The final weights of the residues correspond to the metal oxides as end product [10].

FT-IR Spectroscopy.

Information about the cobalt ion coordination was obtained by comparing the IR frequencies of the ligands with those of the cobalt complexes.

In the figures (fig. 1-3) the main parts of the IR spectra are presented.

In the spectra of the ligands, the ν(N-H) stretching vibrations appear at 3118 cm^{-1} for $\mathbf{L_1}$, at 3052 cm^{-1} for $\mathbf{L_1}$, and at 3146 for $\mathbf{L_3}$. These bands appear to be shifted toward higher frequencies in the spectra of the complexes with 7 cm^{-1} for **(1)**, 250 cm^{-1} and 55 cm^{-1} for **(2)**, and 26 cm^{-1} for **(3)** proving the involvement of the –NH_2 group in the complex formation [11].

The ν(O-H) stretching vibrations do not appear in the ligand spectra, but they do in spectra of their complexes at 3447 cm^{-1}, 3421 cm^{-1} and 3419 cm^{-1} respectively, suggesting the presence of the crystal and coordinated water in these compounds.

The absorption band at 1624 cm^{-1} was attributed to the ν(C=O) stretching vibration in the $\mathbf{L_1}$ spectrum and appears to be shifted to 1637 cm^{-1} for complex **(1),** at 1608 cm^{-1} in the $\mathbf{L_2}$ spectrum and at 1639 cm^{-1} for complex **(2)**, respectively. The same vibration appears in the $\mathbf{L_3}$ spectrum at 1610 cm^{-1} which is shifted with 30 cm^{-1} toward higher frequencies in the spectrum of complex **(3),** displaying a well-resolved and high-intensity signal, which involves the carboxylic group in covalent bonding to the cobalt ion [12].

The consecutive bands at 1600 and 1527 cm^{-1}, in the spectrum of the $\mathbf{L_1}$ were assigned to the symmetric and asymmetric bending vibrations of N-H bond. In the spectrum of the complex **(1)** this band appears at 1559 cm^{-1}, shifted compared to those of the ligand, which means

that $-NH_2$ group is involved in metal-ligand formation. These vibrations in the $\mathbf{L_2}$ and $\mathbf{L_3}$ spectra appear at 1577 and 1510 cm^{-1} and at 1580, 1563 and 1508 cm^{-1}, respectively and are shifted to 1578 cm^{-1} and 1584 cm^{-1} in the coresponding spectra of the complexes **(2)** and **(3)**.

The band at 2904 cm^{-1} in the spectrum of complex **(3)** was attributed to the CH_2-S and CH_3-S bonds and is slightly shifted compared to that of the ligand (2915 cm^{-1}) which means that these groups were not involved in the coordination [13].

UV-VIS Spectroscopy

The bands in the range 200-370 nm can be assigned to $n\rightarrow\pi^*$/ $\pi\rightarrow\pi^*$ intraligand transitions associated to amino acid [14]. Free ligands and complexes exhibit similar spectra in UV region in relation to the number of the absorption bands. A common feature of these spectra is the presence of three absorption peaks. The two located at lower frequencies have been assigned to $\pi\rightarrow\pi^*$(~330 nm) and $n\rightarrow\pi^*$(~260 nm) transition and the additional peak found at higher frequencies corresponds to the $\pi\rightarrow\pi^*$(~210 nm) transition in a sequence of increasing energy. These bands are shifted to lower energy in the cobalt complexes at ~380 nm, ~275 nm and ~225 nm, respectively [15].

The visible spectra of the cobalt complexes exhibit a d-d broad band at 550-450 nm, such a feature should be expected for a octahedral CoO_4N_2 chromophore and can be assigned to $^4T_{1g}(P)\rightarrow{}^4T_{1g}(F)$ transition (fig. 4). Moreover, the variation of the position of the above absorption band can be ascribed to perturbation energies arising from the inductive and delocalization effects of the substituents on the amino acids fragments.

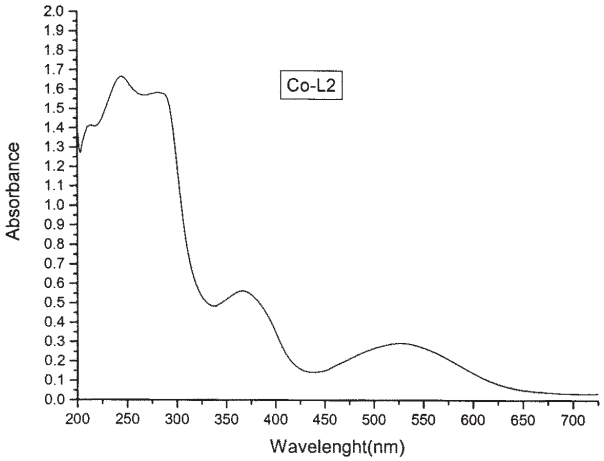

Fig.4. UV-Visible Spectra of **(2)** in water

EPR Spectroscopy

Powder EPR spectra (fig. 5) at room temperature are typical for monomeric species with octahedral local symmetry around the metal ion and are strongly affected by noise. The principal values of the g tensor are: g= 2.20, **(1),** g= 2.16, **(2)** and g = 2.13, **(3)** [16].

Biological Activity

All the amino acids had an increased proliferation rate, but in different proportions, due to the concentrations of the complex or the free amino acid. Generally, copper complexes have similar proliferation rate with the corresponding amino acids. Cobalt complexes, especially Co-leucine (fig. 6) have a proliferation rate increased with 50%. In this experiment it couldn't be established a relation dose-effect, but in general the supplementation with 10 µL/mL of 10^{-4} M amino acids complexes solutions in culture

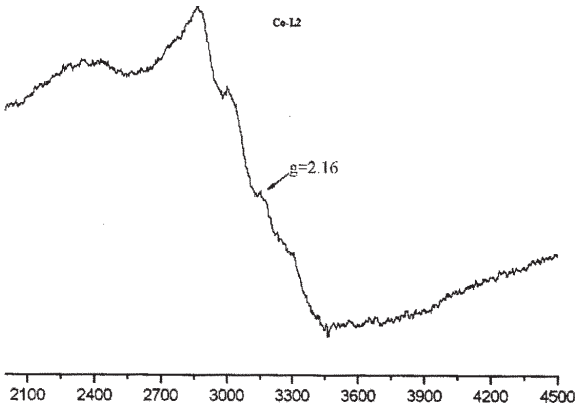

Fig. 5. Powder EPR spectra of **(2)** at room temperature

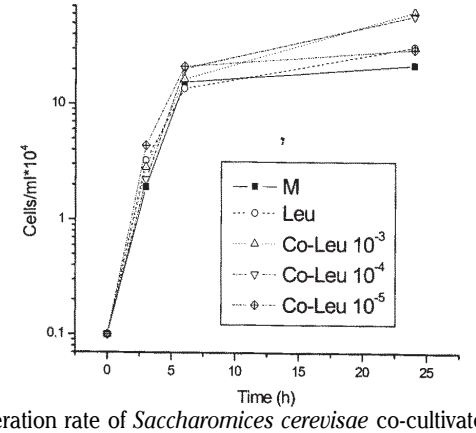

Fig.6. Proliferation rate of *Saccharomices cerevisae* co-cultivated with Co-leucine at three different concentrations

medium offer a good correlation with the tested compound. Leucine complexes with both metals have the higher proliferation rate.

Lactate dehydrogenase (LDH) can be used in cytotoxicity studies, as a marker of cell damage. The normal plasma membrane is impermeable to LDH, but damage of the cell membrane results in a change in the membrane permeability and subsequent leakage of LDH into the extracelular fluid. In vitro release of LDH from cells provides an accurate measure of cell membrane integrity and cell viability. LDH activity is the most used test and reliable for cytotoxicity. Level of LDH from extracelular medium is expressed in nm NADH/min/10000 cells. Because the level of LDH was under 0.15 nm NADH/min/ 10000 cells it can be concluded that the compounds are not cytotoxic.

Lipid peroxidation is the oxidative deterioration of polyunsaturated fatty acids with the production of lipid hydroperoxides, conjugated diene, cyclic peroxides and finally fragmentation to ketones and aldehydes (including malondialdehyde – MDA-).

For all the cobalt complexes the peroxidation level was very low (under 0.1 nmol MDA/mL solution) which means that these complexes have not a peroxidant effect.

It can be concluded that both cobalt-amino acids complexes after three hours increase the proliferation rate in different proportion. After 8 h the inhibition rate for the cobalt-lysine was I_8=1% in average for all three doses and for cobalt-methionine I_8=2% (10^{-3} M), I_8=4% (2.10^{-3} M), I_8=4 % (3.10^{-3}M). After 24 h the inhibition rates for the tested complexes were: cobalt-lysine I_{24}=9% (10^{-3}), I_{24}=13% (2.10^{-3}), I_{24}=13 % (3.10^{-3}M); and for cobalt-methionine I_{24}=8% (10^{-3}), I_{24}=13% (2.10^{-3}), I_{24}=14 % (3.10^{-3}M).

The cellular growth inhibition percents after 24 h are higher in all cases, meaning that these cobalt complexes with amino acids, especially those with methionine, are

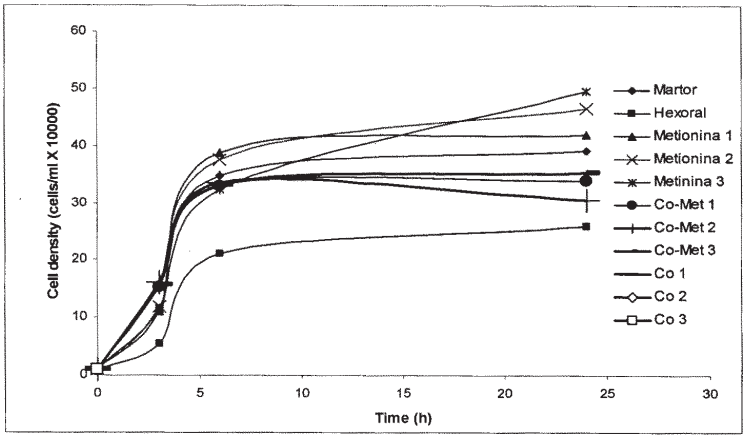

Fig. 7. Proliferation rate of co-cultivated cells culture with Co-methionine at three different concentrations

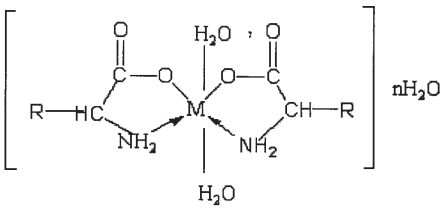

Fig. 8. Molecular formula proposed for the cobalt amino acid complexes

M= Co^{2+}
R = - $(CH_2)_4$-NH_2 for Co-lysine
R = - $(CH_2)_4$ for Co-leucine
R = - $(CH_2)_2$-S-CH_3 for Co-methionine

more efficient against *Candida albicans* on a long term (fig.7), comparing with Heroral, whose inhibition decreased in time.

Conclusions

The cobalt amino acids complexes $[Co(\mathbf{L}_1)_2(H_2O)_2]\times H_2O$ ($\mathbf{L}_1$ =lysine), $[Co(\mathbf{L}_2)_2(H_2O)_2]\times H_2O$ ($\mathbf{L}_2$= leucine) and $[Co(\mathbf{L}_3)_2(H_2O)_2]\times 2H_2O$ ($\mathbf{L}_3$= methionine) were synthesized in aqueous solution and analyzed by means of elemental analysis, thermogravimetric and differential analysis, atomic absorption, IR, UV-VIS and EPR spectroscopies.

The composition corresponded to a metal:ligand ratio in all the Co(II) complexes was found to be 1:2 .

The IR spectra show that the amino acids act as bidentate ligands with coordination involving the carboxyl oxygen and the nitrogen atom of amino group.

The electronic and EPR spectra confirm octahedral local symmetry for the cobalt ion.

Cobalt complexes especially those with methionine were proved to have the higher activity against *Candida albicans* and *Saccaromices cerevisae*. These complexes are efficient against CA on long term because the higher inhibition of growth rate was after 24 h.

The obtained structural data allow us to propose the following molecular formula for the studied cobalt complexes as shown in figure 8.

Other combinations of Cu(II) were studied in previous papers [17-19].

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