Synthesis, Characterization, Solution Stability and Biorelevant Studies of Cobalt (II)–2-Deoxy-D-Glucose Complex Compound

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This paper presents the synthesis of a new complex compound of Co(II) with 2-deoxy-D-glucose (L). This compound was characterized by elemental chemical analysis, electronic and infrared spectra and formulated as binuclear species with the formula, $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$. The complex stability in aqueous solution was investigated by UV-Vis spectroscopy and sustained by FTIR spectroscopy. The biological activity of the as-synthesized complex compound was studied on Pseudomonas aeruginosa ATCC 27853, Aspergillus niger, Fusarium oxisporum and Candida scotti microorganisms.

Keywords: Co(II), biological activity, 2-deoxy-D-glucose, complex compound

Cobalt is an essential element which influences many enzymatic systems and is found in small amounts in most body tissues, with the highest concentration in the liver [1]. Gastrointestinal absorption from food or water is the principal source of internally deposited cobalt in the human body. Vitamin B12 is a cobalt-containing vitamin with a high intestinal absorption, which is essential for red blood cells formation in humans.

Saccharides compounds are widespread in nature and their biological role is well known. Tests made on rats have shown that dietary supplementation with 2-deoxy-D-glucose improves cardiovascular and neuroendocrine stress adaptation and glucose metabolism by decreasing the concentrations of blood glucose and insulin under non-stress conditions [2]. Treatment with 2-deoxy-D-glucose mimics the beneficial effect of dietary restriction *in vivo* and protects cultured dopaminergic cells against oxidative stress, metabolic trauma relevant to the pathogenesis of Parkinson's disease and preserved mitochondrial function [3]. The interactions between metal ions that are normally found in human body and saccharides represent an area of interest for various potential applications such as biotechnology and pharmacology.

This study presents the synthesis of a complex compound containing Co(II) and 2-deoxy-D-glucose (L). The characterization of $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ was done by elemental analysis, FTIR and UV-Vis spectroscopy, thermal analysis TG-DSC, molar electrical conductivity as well as magnetic measurements. The stability in aqueous solution and toxicity of the complex were also investigated.

Experimental part

Sigma Aldrich supplied all reagents and solvents.

Synthesis of Co(II)-2- deoxy-D-glucose complex compound: 2-deoxy-D-glucose (L) was used ($C_6H_{12}O_5$) (3 mmol, in 100 mL methanol) as sodium salt generated by adding metallic sodium (18 mmol) in small pieces. After 45 min a methanolic solution (33mL) of $CoCl_2 \cdot 6H_2O$ (3 mmol) was added slowly with constant stirring to the foregoing mixture at room temperature to give a violet precipitate that changed in brown in 6 h. The reaction mixture was stirred for 24 h. in order to ensure the completion of the reaction. The precipitate was isolated by filtration and then was purified by stirring four times with a mixture of 9:1 methanol-water and finally with methanol. The product was dried under vacuum. *Anal* Found (%): C, 22.50; H 4.72, Na, 3.61; Cl, 16.35; Co 18.40. Calculated for $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ (%): C, 22.33; H 4.68, Na, 3.56; Cl, 16.48; Co 18.26.

The metal content of the complex compound was determined by atomic absorption spectroscopy with a SAA1 instrument and by gravimetric techniques; the C and H values were obtained using a Carbo Erba Model 1108 CHNSO elemental analyser. The chlorine content was determined by gravimetric method. The sodium content was determined by Flame Emission Photometry method.

UV-Vis spectra measurements were done with a JASCO V560 spectrophotometer with solid sample accessory, in the domain 200-800 nm, with a speed of 200 nm·min^{-1}.

The FTIR spectra were recorded on KBr pellets with a Bruker Tensor 27 spectrometer in the 4000–400 cm^{-1} range.

The thermal decomposition of the compounds was followed with a Netzsch 449C STA Jupiter. Samples were placed in open alumina crucible and heated with 10°C min^{-1} from the room temperature to 800°C, under the flow of 20 mL min^{-1} dried air.

Molar electrical conductivity have been measured in 10^{-3}M DMF solutions, at 25°C, with OK 102/1 Radelkis Conductometer, 0.1-0.5 S. Λ_M obtained for $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ was 75Ω^{-1}cm^{2}mol^{-1} assigned to 1:1 electrolyte type.

The magnetic measurements were performed using Faraday's method, at room temperature.

For the biological tests, the disk diffusion method of antimicrobial susceptibility test was adapted to determine the toxic properties of prepared Co(II) complex compounds with 2-deoxy-D-glucose. The experiments were accomplished in Petri plates containing Mueller Hinton agar (for bacteria) or 2% Sabouraud glucose agar (for fungi) previously inoculated with microorganisms cultures (suspension of microorganisms culture in 1 % sterile peptone water, resulted from fresh cultures selective media of microorganisms with 10^8 cfu·mL^{-1} corresponding to 0.5 McFarland standards in turbidity).

The Muller–Hinton agar was obtained by dissolution of 3g beef extract, 17.5g casein acid hydrolysate and 1.5g starch in 1000 mL distilled water, followed by sterilization in autoclaved at 116-121°C for 10-15 min and then the mixture was left to solidify. Sabouraud 2% glucose broth was prepared by dissolution of 10g mycological peptone and 20g glucose in 1000 mL distilled water, followed by

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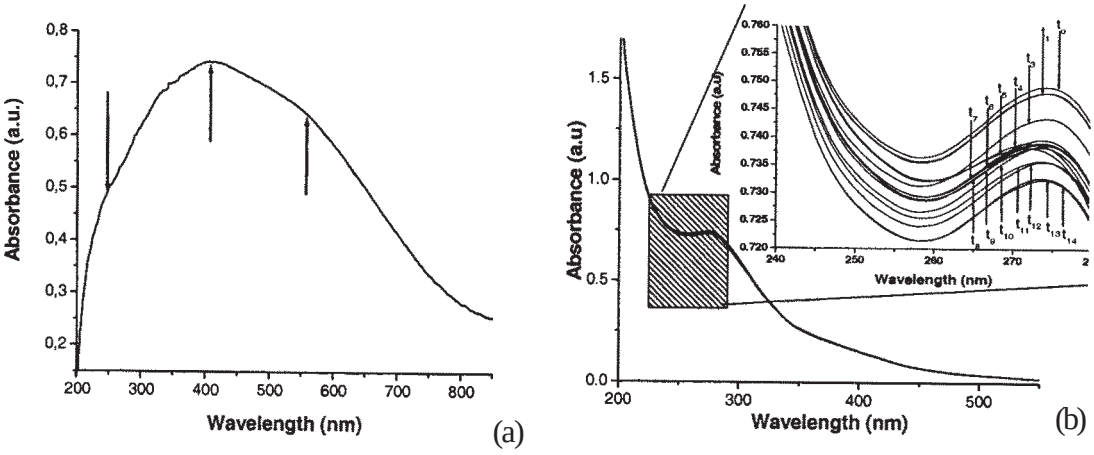

Fig.1. UV-Vis spectra for $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ complex in solid state (a) and in aqueous solution during 12 h (b) inset the detailed 240-280 nm range of absorbance in time t_1= 60 min, t_2= 120 min, t_3= 180 min, t_4= 240 min, t_5= 300 min, t_6= 360 min, t_7= 420 min, t_8= 480 min, t_9= 540 min, t_{10}= 600 min, t_{11}= 660 min, t_{12}= 720 min, t_{13}= 780 min, t_{14}= 840 min

sterilization in an autoclave at 121°C for 15 minutes and solidification at room temperature.

The surfaces of the agar Petri plates were inoculated with microorganisms strains by streaking the swab over the entire sterile agar surface. In the Petri plates were inserted 50, 25, 16 and 12 mg/mL concentration of $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ complex placed at equal distances (3 cm from the centre Petri plate and 1.5 cm from the edge of the plate). The Petri plates were placed in an incubator at 37°C for 18 - 24 h in the case of bacteria and 24 - 48 h for fungi. The diameter of the inhibition zones was measured after incubation and compared with those of the standard antibiotics with the following concentrations (Tobramycin 3mg/disc, Erythromycin 1.5mg/disc, Clindamycin 1mg/disc, Ciprofloxacin 5mg/disc). Each determination of the complex biologic activity was tested in three replicates.

Results and discussions

UV-Vis spectrometry

UV-Vis spectrum of $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ reveals a broad band on the entire visible and ultraviolet domains obtained probably by superposition of d-d transitions of the two cobalt (II) ions in octahedral symmetry, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(F)$.

Generally, metal-saccharide complexes are expected to have low stability [4]. Dilute solutions of these complexes with concentrations lower than 1.5 mM resulted in their precipitation as hydroxides in 15-30 min, whereas at concentrations higher than 4 mM were stable over a week, with no precipitation [5].

In our case, the absorbance spectra measurements of diluted solution of the complex compound with $1.5x10^{-3}$ M concentration taken during a time period of 14 h did not show any modifications as it could be seen from figure 1b. This stability was also observed in our previous studies with the saccharide complexes [6]

FTIR spectrometry

By comparing the FTIR spectra of the $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ with that of the ligand it can be observed that almost all the vibration modes are shifted or merged resulting a broadening of spectral bands. The merging and broadening of these bands is a common feature of transition metal-saccharide complexes synthesized from the saccharides sodium salts [7]. The free ligand stretching vibrations assigned to νO-H groups in the region 3500-3200 cm^{-1} were transformed upon complexation and become a broad band at ~ 3400 cm^{-1} with a shoulder at ~ 3250 cm^{-1}, in complex compound, indicating a rearrangement of the hydrogen bonding network of ligand upon complexation. In this region the OH vibration of water molecules are overlapped. The peak observed in the ligand spectra at 2800 cm^{-1} attributed to asymmetric C-H stretching vibration was found at 2850 cm^{-1} in $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ spectra.

The stretching vibrations assigned to C-O and C-C presented in the region 1140-990 cm^{-1} were merged at ~ 1040 cm^{-1} upon complex formation, in contrast to the sharp bands observed for the free saccharides and other metal-saccharide adducts. The spectral bands assigned to C-O, C-C, O-CH and C-CH presented in the regions 1600–1650 cm^{-1}, 1350–1450 cm^{-1} and 1000–1100 cm^{-1} in $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ spectra were merged and shifted in comparison with those of free ligand spectra, indicating the presence of metal-saccharide interactions. In the region 400-1000 cm^{-1} were found bands corresponding to Co(II)-O vibrations [8,9].

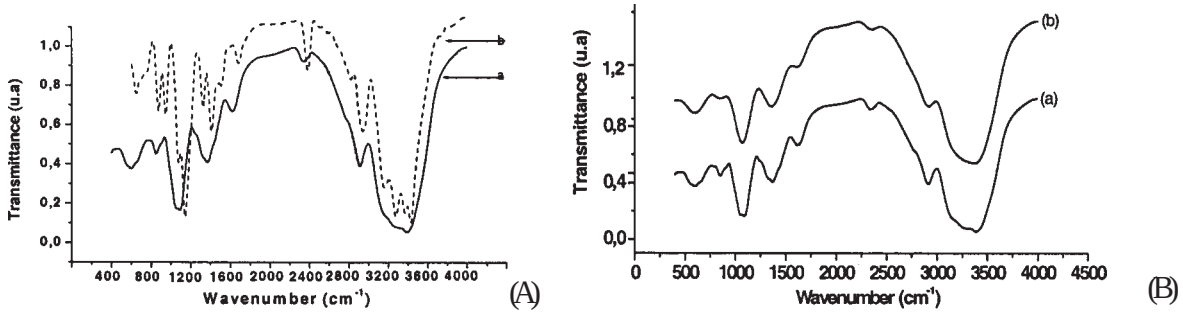

Fig. 2. (A) FTIR spectra of complex compound $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ (a) and 2- deoxy-D-glucose (b); (B) a comparison between the FTIR spectra of $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ before (a) and after aqueous stability investigation (b)

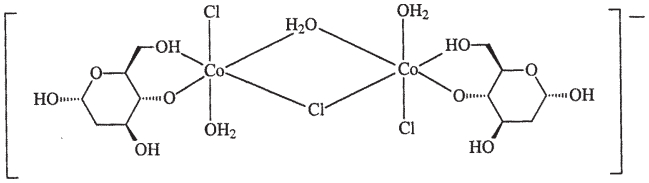

Fig.3. The structural formula proposed for $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ compound

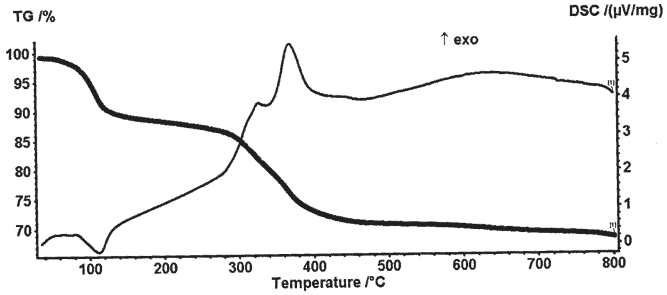

Fig.4. The thermal analysis for $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ complex

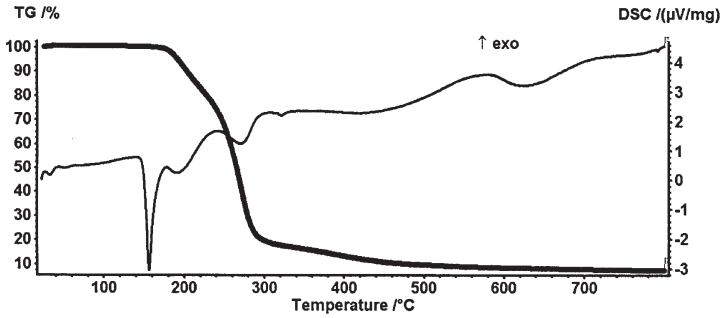

Fig.5. The thermal analysis for 2-deoxy-D-glucose

Therefore, the FTIR spectra support the complex formation indicating the binding of 2-deoxy-D-glucose units to the cobalt ions.

Magnetic measurements

The magnetic behaviour of octahedral high spin complexes is very difficult to be explained because of the orbital angular momentum. Two dominant effects must be taken into consideration. Firstly, the local spin-orbit coupling of each mononuclear unit is strongly influenced by local distortion around each cobalt (II) ion. The second effect is the exchange interaction between Co(II) ions considered an interaction between the effective spins obtained from the spin-spin coupling. In addition, the following factors must be taken into consideration: the large anisotropy of the effective spins (3/2) and the orientation of the distortion axes [10].

The value of magnetic moment determined for $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ at 300K which correspond to a magnetic moment, μ_{eff}, value of 4.405 MB, assigned to a octahedral geometry of Co(II). The magnetic moment value of $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ complex compound is in agreement with the literature [11].

Thermal analysis

The thermal analysis for $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ presents two decomposition steps in the 30-800°C interval (fig.4). The first decomposition process takes place in the interval 80-180°C, corresponding to a mass loss of 11.22%. This process was attributed to four water molecules elimination (calcd. mass loss 11.41%). The process is accompanied by an endothermic effect on the DSC curve.

The second step of decomposition takes place in the interval 260-500°C and has a corresponding mass loss on TG curve of 17.10%. This mass loss was attributed to ligand oxidation process. This process is accompanied by a strong exothermic effect, as expected in an oxidative process.

By comparing the TG curves for the complex compound $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ and ligand (fig.5), one can see that the free ligand decomposition takes place at a lower temperature (180°C) than that of the ligand from the complex (260°C). The enhanced thermal stability of the ligand in the complex can be directly attributed to the formation of the coordinative bonds.

A slow oxidation process of the residual mass, 2.43% mass loss, takes place in the interval 500-800°C, in association with a broad exothermic effect on DSC curve. The residual mass, 68.90%, probably consists of CoO, $NaCoO_2$ and carbonaceous mass.

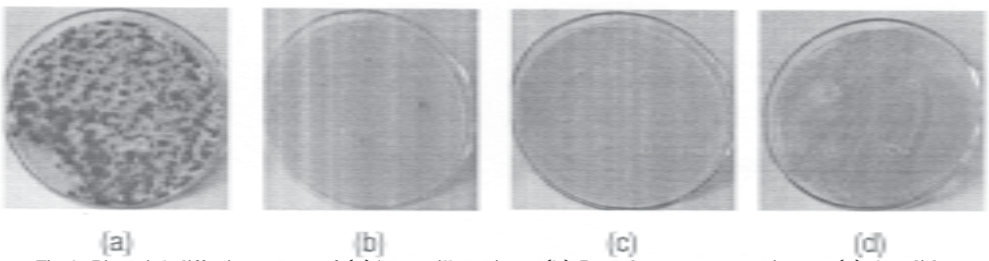

Fig.6. Pictorial diffusion spots of (a)Aspergillus niger; (b) Pseudomonas aeruginosa; (c) Candida scotti; (d) Fusarium oxisporum for 50 mg/mL $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ complex compound

Biological tests

The biological activity experimental data indicated that the $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$ complex in the following range of concentrations 50, 25, 16, 12 mg/mL, did not inhibit Aspergillus niger, Pseudomonas aeruginosa or Candida scotti strains. The strains presented growth characteristics for each tested concentration of the complex (fig.6 a, b and c). In the case of Fusarium oxisporum, the Co (II) complex compound had an inhibitory activity by stopping the sporulation phenomena, presenting only a vegetative growth (fig.6 d).

Conclusions

The synthesis of Co(II)-2-deoxy-D-glucose compound has resulted in the formation of a binuclear complex compound $Na[Co_2(L)_2Cl_3(H_2O)_3]H_2O$. UV-Vis studies, together with magnetic measurements results indicate the presence of octahedral high-spin Co (II). FTIR spectra confirm the coordination of 2 deoxy-D-glucose ligand to Co(II) ions. The weight losses observed by thermal decomposition are in good agreement with the calculated losses based on proposed formula for the complex. The ligand decomposition takes place at a higher temperature in the complex compound than in the case of the free ligand, this thermal stability being a direct consequence of the coordination process.

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