Complex Compounds of Some 3d Ions with 2 Deoxy D Glucose
1. Iron Complex

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A paramagnetic Fe(III)- 2 deoxy D glucose (Fe-dGl) complex compound with $Na_2[Fe_2(\mu OH)_2(2dGlc_2)_2(H_2O)_2Cl_2]$ formula was synthesized and characterized by elemental analysis, IR and UV-VIS spectroscopy, thermal analysis TG-DSC, molar electrical conductivity as well as magnetic measurements and Mössbauer spectroscopy, with the aim of developing a new possible MRI contrast agent. The stability in physiological conditions and toxicity of the complex have also been established.

Keywords: iron, Fe-2 deoxy D-glucose complex compound, MRI contrast agent

The complexation of iron with saccharide and polyol ligands has been extensively studied due to its potential use in the treatment of iron-deficiency anemia [1,2]. In the same time, paramagnetic iron-saccharide complexes could be good contrast agents in cancer diagnosis [3]. For exemple, the most commonly used PET (Positron Emission Tomography) radiotracer for cancer diagnosis is a compound of saccharide type, 2-fluorine-[8]-fluoro-deoxy-D-glucose (^{18}FdG) [4, 5,6].

The design of coordination compounds for use as paramagnetic MRI contrast agents presents a particular challenge to the coordination chemistry. In order to develop a successful agent it is necessary to reconcile several conflicting requirements. The biological requirement is for a compound with low toxicity combined with suitable biodistribution behaviour. Low toxicity is an important issue since relatively large doses are required for a MRI contrast agent to be effective, typically in the range 0.1 – 0.3 mmol kg $^{-1}$ [1]. This makes it particularly important that the metal is not readily released from the contrast agent as this would allow binding to serum proteins or other *in vivo* complexing agents. Transfer of the metal in this way would result in a loss of control over metal ion toxicity and biodistribution. Rather the complex should remain intact and be excreted completely and rapidly following the imaging procedure. This requires the complex to be thermodynamically stable and kinetically inert. The basic requirements for an effective contrast agent are that it should have a high magnetic moment, accommodate at least one water molecule in the first coordination sphere of the metal ion and that the coordinateion water to be quickly replaced with the water from agglomerate. In order to attain a high magnetic moment it is necessary to choose high spin metal ions with a large number of unpaired electrons [4].

In this paper we reported the synthesis, characterization and biological tests of a complex compound with a saccharide type ligand iron (III) 2 deoxy D glucose (Fe-dGl), that can be applied as a MRI contrast agent.

Experimental part

Synthesis of Fe(III)-2 deoxy D glucose (Fe-dGl) complex compound:

2 deoxy D glucose ($C_6O_{12}O_5$) (9 mmol, 1.35 g) was dissolved in 60 mL methanol to which sodium (0.414 g,18 mmol) was added in small pieces. To this, 40 mL of methanolic solution of ferric chloride (0.486 g, 3 mmol) was added to give a yellow precipitate. After stirring for 3 h, a brown colored solid was obtained. The reaction mixture was stirred for 24 h in order to ensure the completion of the reaction. The solid was obtained by filtering the reaction mixture and further washing with ether and drying under vacuum. The isolated product was purified by dissolving it in a 2:1 water-methanol mixture and the product was reprecipitated with ethanol. The purification process was repeated three times. The product thus obtained was washed with ether and dried under vacuum. *Anal* Found (%): C, 22.7; H, 3.90; Na, 7.25; Cl, 11.15; Fe, 17.65. Calculated (%) for $Na_2[Fe_2(\mu OH)_2(2dGlc_2)_2(H_2O)_2Cl_2]$ ($C_{12}H_{24}Cl_2Na_2Fe_2O_{14}$):C, 22.53; H, 3.85; Na, 7.19; Cl, 11.11; Fe, 17.52.

All reagents and solvents were purchased from Sigma Aldrich.

The final complex compound was investigated by elemental chemical analysis. The electronic spectra was recorded on a Jasco V-550 Spectrophotometer. The FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer using KBr as reference, in the range of 4000-400 cm^{-1}.

Thermal analyses were performed using a NETZSCH Proteus 449C thermal analysis with alumina crucible in Ar atmosphere. Selected Ramp rate was of 10^{0}C/min to 900^{0}C in the range 20-900^{0}C.

A Shimadzu diffractometer with CuKa radiation was used to analyze the crystallographic phases of the calcined samples from thermal analysis.

Molar electrical conductivity has been recorded in 10^{-3}M DMF solutions at 25^{0}C, with OK 102/1 Radelkis conductometer with a 0.1 S – 0.5 S measuring range. Λ_M (Ω^{-1}cm^{2}mol^{-1}) was found to be 70Ω^{-1}cm^{2}mol^{-1} corresponding to 1:1 electrolyte type.

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

The Mössbauer spectra were recorded at room temperature in the standard transmission geometry, using a 25m Ci ^{57}Co in Rhodium matrix and a Wiesel data acquisition system. The velocity calibration was performed with a standard alpha iron foil.

For the biological tests the diffusion method of antimicrobial susceptibility test was adapted to quantify toxic properties of the Fe 2 deoxy D glucose, $Na_2[Fe_2(\mu OH)_2(2dGlc_2)_2(H_2O)_2Cl_2]$. Basically, Fe-dGl complex compound impregnated paper disks is stuck

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firmly on the surface of a culture appropriate medium that has been previously inoculated with pure microorganisms stains suspension.

Inocula preparation

A standard young strain of *Pseudomonas aeroginosa* tulpine ATCC 15445, *Aspergillus niger, Fusarium oxisporum*, and respectively *Candida scotti* were selected to test the toxic properties of the compound. Each selected culture was spread onto a non inhibitory agar medium to obtain isolated colonies. After some days of incubation, well-isolated colonies were collected before sporulation or conidia growth to avoid the outset vegetative forms which are more sensitive and do not yield confident results in such trials. Specimens of these inoculates were suspended in sterilized water, shaken for homogenization until an opalescent fluid is substantiated and additionally left for 10 min for air separation. The suspension should then be compared to the 0.5 McFarland standard. Consequently, the opalescent fluid is poured in small drops under continuous mixing into a tube containing 3 mL of sterilized water and some glass beads until the sample reach the opalescence of the 0.5 McFarland turbidity standard corresponding to $0.4x10^4$- $5x10^5$ CFU/mL for *Aspergillus niger* and *Fusarium oxisporum* and 1.8 x 10^4 for *Pseudomonas aeroginosa* and *Candida scotti*.

Results and discussions

FT-IR spectra

Generally, the transition metal-saccharide complexes show broad FT-IR spectra [2,6]. The ν(O-H) bands of the free saccharide are observed in the region 3500-3200 cm^{-1}, which upon complexation, show merging, exhibiting a broad band at 3400 cm^{-1}. Further, the resolution of this band is poor due to the overlap of ν(O-H) of water molecule. The ligand ring vibrational frequencies for the bending modes of δ(OCH, CH_2 and CCH) of the free saccharide ligand 1460-1340 cm^{-1} showed merging at 1400 cm^{-1} upon complex formation. The bands observed between 2920 and 2940 cm^{-1} are assigned to ν(C-H) also indicating the presence of saccharide moieties in the product [16]. The ν(C-O) and ν(C-C) stretching vibrations in the region 1140-990 cm^{-1} were also merged to give a broad band at 1050 cm^{-1} upon complexation, in contrast to the sharp bands observed in case of the free saccharides [7]. Yet another evidence for the presence of water molecules may also be derived from the δ(H-O-H) bending vibrations observed at 1603 cm^{-1} in Fe-dGl complex studied. The complex exhibited bands in the range 509-519 cm^{-1}, assignable to ν_{sym}(Fe-O) vibrations based on literature comparisons [5,8,9].

Thus the FTIR spectra have clearly indicated the binding of saccharide moieties to Fe(III) centres.

UV-VIS spectroscopy and Solution stability

The reflectance spectrum of the complex in the solid state presents an intense band in UV range at about 280-300 nm characteristic for the free ligand. In general, *d-d* bands corresponding to a FeO_6 chromophor have extremely low intensity as a consequence of their doubly forbidden spin transition nature for a $3d^5$ electron configuration [10].

The spectrum of $Na_2[Fe_2(\mu OH)_2(2dGlc_2)_2(H_2O)_2Cl_2]$ complex presents a band around 370-390 nm which is consistent with the presence of high-spin iron(III) centre and the complexation of saccharide through deprotonated hydroxyl groups [2,7]. The compound is highly soluble in water and can be solved in MeOH and N,N-dimethyl formamide. The spectra of the complex in these solvents are shown in figure 1, and the corresponding data are given in table 1.

The similarity of the diffuse reflectance spectra and the aqueous solution absorbtion spectra of the Fe-dGl complex suggests their structural integrity in the solid state as well as in the freshly prepared solution.

The two bands observed in the region 250-390nm correspond to oxigen → metal charge transfer transitions and the weak shoulder observed around 470 nm is characteristic for octahedral, high-spin d^5 complex [8]. The 470 nm shoulder showed marginal shift in MeOH and N,N-dimethylformamide. The marginal changes observed in the absorption spectra among the three solvents may suggest the involvement of solvent molecules in the coordination sphere [7].

This is in accordance with our findings based on FT-IR, TG-DSC and elemental analyses that H_2O molecules are indeed involved in the inner sphere of the complex.

Compound Fe(III)-dGl is stable in physiological conditions (*p*H = 7.2) during 24h as it can be seen from figure 2B.

Magnetic measurements

The effective magnetic moment value for Fe(III)- 2dGl was found to be 5.0 BM at 298 K, a value that is comparable with that expected for a paramagnetic dinuclear, high-spin Fe(III) complexes. This low value of effective magnetic moment indicates the presence of some magnetic interaction between the iron centers [10]. Based on a large number of compounds reported in the literature it is noted that μ-OH compounds have μ_{eff} in the range 4.0-5.3 BM [2,11].

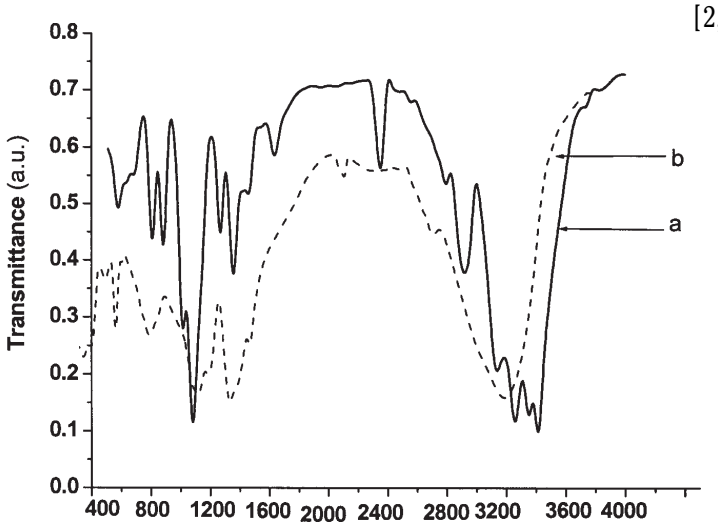

Fig 1. FT-IR spectra of 2 deoxy D-glucose (a) and complex compound Fe-dGl (b)

Table 1
SOLUTION UV-Vis dATA FOR IRON -2 DEOXY D GLUCOSE AS MEASURED IN H_2O, MEOH AND N,N-DMF

Compound	λ (nm, ϵ, $cm^{-1}mol^{-1}$)		
1	H_2O	MeOH	N,N-DMF
	220(6708), 290(18632), 470(535)	211(6653), 300(3948), 470(118)	265(5133), 330(2965), 470(77)

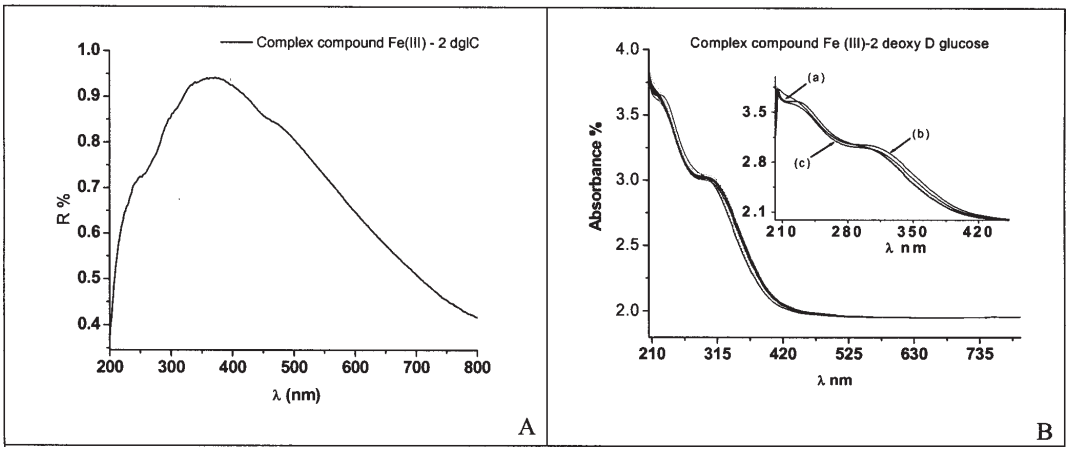

Fig.2. UV-Vis absorbtion spectra for complex Fe-2 deoxy D glucose solid state (A) and in aqueous solution during 24 h (B)inset the variation of absorbance in time after 6 h (a); 12 h (b) and 24 h (c)

Mössbauer Measurements

In fig. 3 the Mössbauer spectrum of the sample Fe(III)-2dGl is presented together with the computer fit (continuous line in fig. 3) in the hypothesis of Lorentizan lineshape. Mössbauer spectrum of the sample Fe 2D consists in a quadruple pattern with isomer shift (IS) of 0.31 mm/s and quadrupole splitting (QS) of about 0.79 mm/s. The IS values in Mössbauer spectroscopy make it possible to draw information about the character of chemical bond, valence state, coordination etc. The QS is a result of the interaction between the quadrupole moment of non-spherical Mössbauer nucleus and the electric field gradient created at the nucleus by the electron shell and ligands, bearing information on the structure of the atomic environment of the nucleus in the crystal lattice and on the state of its valence shells. In our case, the IS and QS values given by the computer fit support the presence of Fe III in a distorted octahedral symmetry [12]. No other phases or inequivalent iron positions in the lattice can be inferred from the obtained fit result of Mössbauer spectrum.

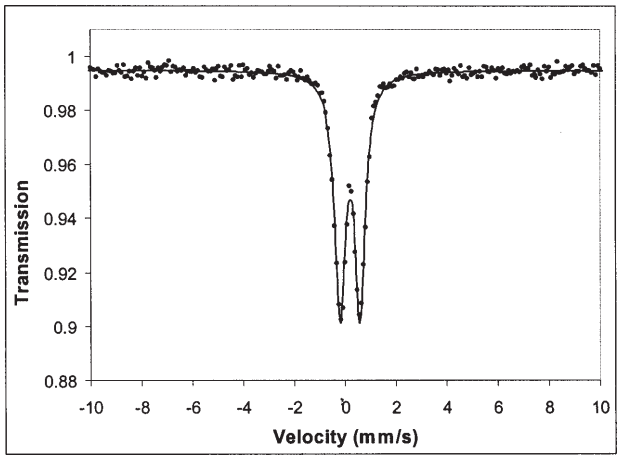

Fig.3. Mössbauer spectrum for Fe(III)- 2dGl

Based on all the experimental data and HyperChem molecular modeling of the ligand (fig. 6.a), we propsed the following structure for the compound Fe-dGl (fig.6.b).

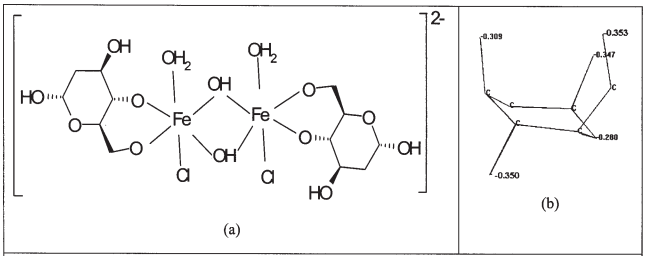

Fig. 4. The proposed structure for complex compound Fe(III)-dGl (a) and for the ligand 2 deoxy D glucose ligand (b)

Thermal analysis

Thermal decomposition of the synthesized compounds Fe-dGl has been recorded in order to investigate their thermal stability and to confirm the proposed formulae. The temperatures of decomposition suggest that the complex is thermally more stable (fig.5.a) comparing to the ligand (fig.5.b) due to coordination [13,14].

The first step of complex decomposition in the range 40-140^{0}C belongs to the coordination water molecules loss associated with an endo effect (-2H_2O=7.46%). The combustion of the saccharide ligand began between 140-260^{0}C and is attributed to acetone and CO (-CH_3COCH_3, --CO = 11.45 %). In the 260-340^{0}C and 340-560^{0}C ranges the combustion is completed with elimination of the volatile molecules acetone and CO producing exothermic and endothermic effects (-2CH_3COCH_3, -CO = 23.74%). The remained products, Na_2CO_3, NaCl and Fe_3O_4 (magnetite) are transformed in NaCl-Fe_3O_4 between 560-900^{0}C by losing CO_2 (-CO_2 =7.16 %) associated with endothermic effect (fig.5.a). The X-ray pattern of Fe(III)-dGl complex calcined at 750^{0}C correspond to a Fe_3O_4 (magnetite) (ICDD 82-1533) and NaCl (ICDD 78-0751) mixture.

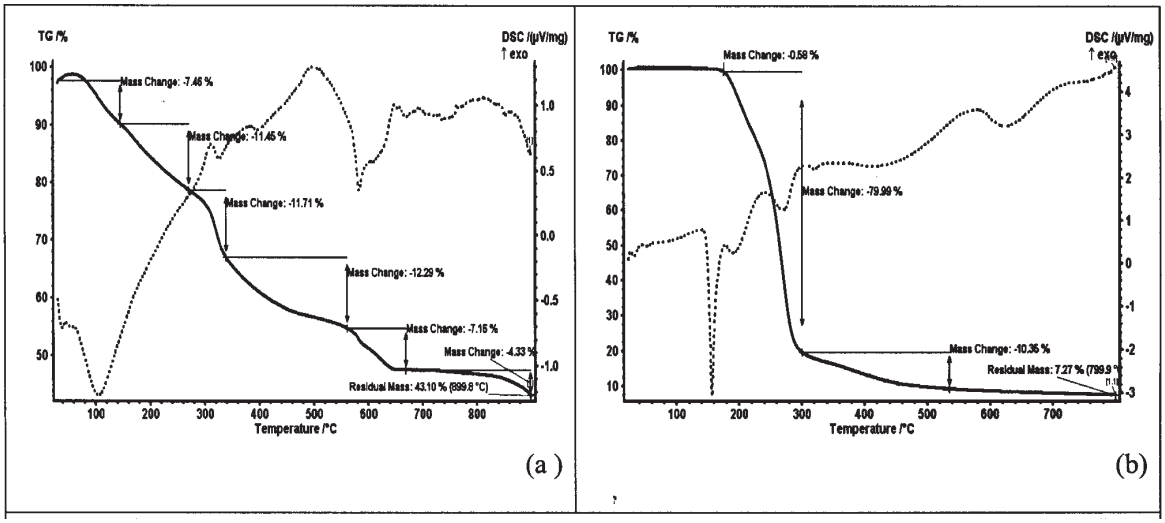

Fig 5. Thermal analysis TG/DSC of complex compound of Fe(III) (a) and 2 deoxy D glucose (b)

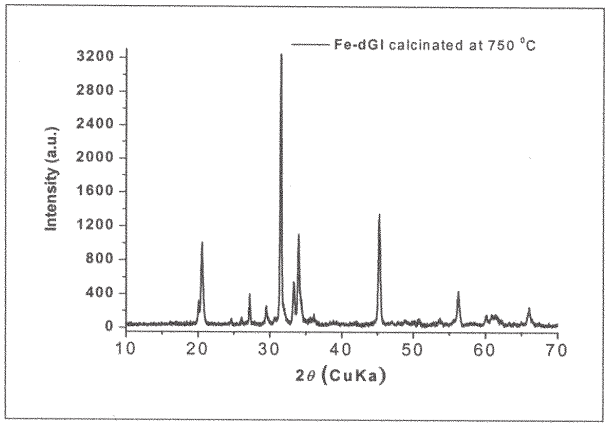

Fig.6 . XRD pattern of the Fe-dGl complex calcined at 750 ^{0}C

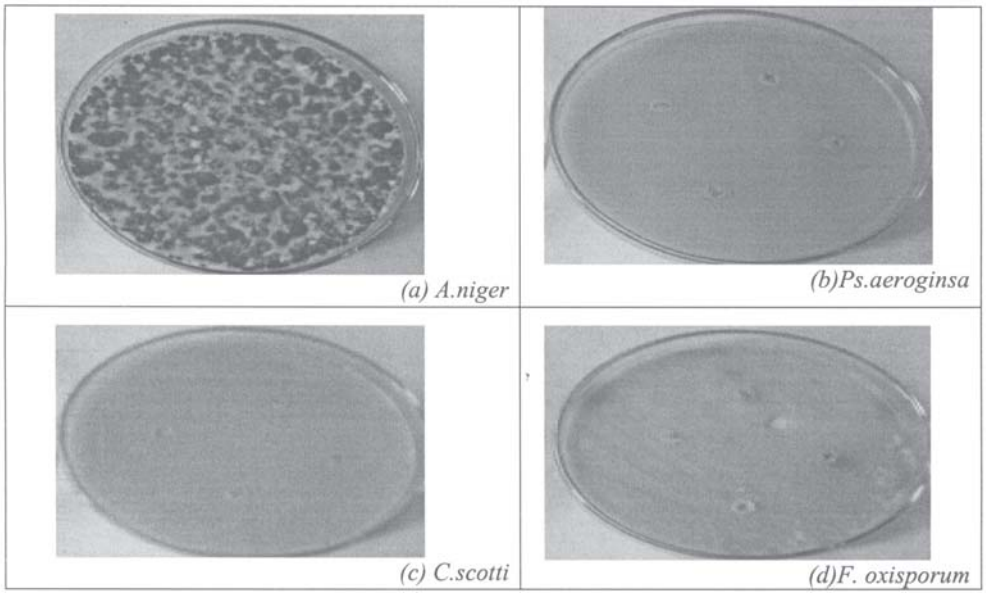

Fig.7. Pictorial diffusion spot of (a)Aspergillus niger; (b) Pseudomonas aeroginosa; (c) Candida scotti ; (d) Fusarium oxisporum; after addition of 50 mg/mL Fe (III)- dGl complex

Biological response of the complex Fe(III)- dGl

The experimantal data indicates that *Aspergillus niger, Pseudomonas aeroginosa, Candida scotti tulpines* were not inhibated by the Fe(III)-dGl complex in the range of concentration 50, 25, 16, 12,6 mg/mL. The *tulpines* presented growth characteristics for each concentration of the complex tested (fig. 7. a,b,c)

In the case of *Fusarium oxisporum,* Fe(III)-dGl had an inhibition effect by stopping the sporulation phenomena, presenting a vegetative growth (fig. 7.d).

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Conclusions

A new possible contrast agent has been prepared based on the requirements necessary for MRI. The structure of the newly synthesized compound has been proposed based on elemental analysis, FT-IR and electronic spectra,

thermal TG-DSC analysis, conductometric, magnetic and Mössbauer measurements. The biological tests and stability in physiological conditions were also investigated. The basic requirements for a MRI contrast agent were accomplished.

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