SYNTHESIS, CHARACTERIZATION AND BIOLOGIC ACTIVITY OF NEW COMPLEXES OF 3,6-DIAMINOACRIDINE (PROFLAVINE) WITH 3*d* AND 4*f* METALS♦

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Abstract: In this paper we report the preparation, characterization and antibacterial activity of some new complex compounds of 3,6-diaminoacridine (L) with 3*d* - Fe(III) and Cu(II) and 4*f* - Gd(III) and Ce(III) metals. On the basis of chemiluminescence properties studies of complex compounds antioxidative character of these complex compounds is discussed.

Keywords: *complex compounds, lanthanides, antibacterial activity*

INTRODUCTION

In clinical chemistry, most of the compounds of interest are present in the body fluids at concentration so low that common analytical methods are not efficient for their determination. Yalow and Berson [1] proposed to determine these products using

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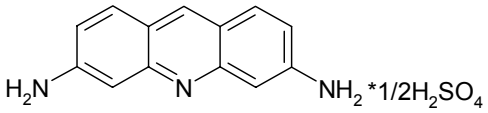
antibody antigen reactions after radiolabeling of one of the partners, the antigen (insulin) in order to discriminate bound and free components. The widespread opinion that radioactive labels are unsuitable for non-separative protocols and the known drawbacks of radioisotopes increases the need for compounds allowing non-isotopic detection although homogeneous immunoassays have been described, using low-energy as well as high-energy radioisotopes, since the past-decade [2,3]. Luminescence and especially chemiluminescence is one of these alternatives.

The aim of this work is to present the isolation, characterization and biological properties studies of some complexes of proflavine (3,6-diaminoacridine) **L** with iron(III), copper(II), gadolinium(III) and cerium(III).

Elemental chemical analysis, molar electric conductivity data and infrared spectra support the proposed formulae for these complexes:



L

EXPERIMENTAL

L (3,6-diaminoacridine) and the Ce(III), Gd(III), Fe(III) and Cu(II) salts [$Ce_2(SO_4)_3 \cdot 4H_2O$, $GdCl_3 \cdot 6H_2O$, $FeCl_3$ and $CuSO_4 \cdot 5H_2O$] were purchased from *Fluka* and respectively *Merck* and used without further purification.

The complex compounds were obtained by 10 hours refluxing of methanol solutions of cerium, gadolinium, iron and copper salts and proflavine in corresponding molar ratio of 1:1. All the compounds were washed with ethyl ether and then dried slowly at room temperature. The precipitates were dissolved in warm methanol and after the evaporation of the solvent the complexes were dried in a dessicator over $(P_2O_5)_2$. All the reactions are quite facile and the resulting complexes could be isolated with 65-73% yields.

Nitrogen was analysed by microcombustion Dumas. Chlorine and sulfur ions were determined gravimetrically. Molar electrical conductivities were determined in acetonitrile (CH_3CN) solutions at 25 °C with OK 102/1 Radelkis Conductometer.

Luminiscence spectra were carried out on TD 20/20 Turner Designes instrument at 430 nm with Luminol (LH_2 = 5-amino-2,3-dihidro-1,4-phtalazindione) in H_2O_2 medium as reference sample.

The biological activity of the complexes and ligand was tested comparative in solid medium on different bacterial strains by diffusion method on Petri plaque. The substances were impregnated on Millipore paper microdisk with solution (200

μg/microdisk concentration). By measuring minimum inhibition diameter of the bacterial growth the "bactericide power" of the tested substances was established [4, 5].

RESULTS AND DISCUSSION

The new complex compounds are isolated as brown powders and characterized by elemental chemical analysis, molar electric conductivity data and infrared spectra. The proposed formulae for the synthesized complexes are $[Ce_2L_2(SO_4)_3(H_2O)_8]_n$ – ***A***, $[GdLCl_3(H_2O)_5]$ – ***B***, $[-Fe_2L_2Cl_5(H_2O)_2-]Cl$– ***C*** and $[CuL(SO_4)(H_2O)]$ – ***D***.

The molar conductance measurements, performed in acetonitrile solution 10^{-3} M, indicate that all complexes have a non-electrolyte behaviour (Table 1).

Table 1. *Elemental analysis results and molar conductivity data*

Compound	M (g/mol)	% N		% S/Cl		Λ^* [μS/(cm·mol)]	Electrolyte type
		Calcd.	Exp.	Calcd.	Exp.		
$[Ce_2L_2(SO_4)_3(H_2O)_8]_n$ – A	1130	7.43	7.67	8.49	8.72	49	Non-electrolyte
$[GdLCl_3(H_2O)_5]$ – B	611.5	6.86	6.98	17.41	17.6	14	Non-electrolyte
$[-Fe_2L_2Cl_5(H_2O)_2-]_n$ – C	779	10.8	1.24	27.34	27.56	128	Electrolyte 1 : 1
$[CuL(SO_4)(H_2O)]$ – D	436	9.63	8,87	11.01	10,79	17	Non-electrolyte

*10^{-3}M acetonitrile solutions

Infrared spectra

The coordination sites of the ligand involved in bonding with the metal have been determined by careful comparison of the IR spectra of the complexes with those of the ligand. Since the full spectrum of the ligand is highly complex, only those bands that are useful for diagnosis of coordination with the metal ion have been taken for discussion.

The shift to lower energy and splitting of amine group stretching bands, δ_{N-H} proved that in all complexes acridine L is coordinated by the nitrogen atom acridine: δ_{N-H} in complexes are 1637 cm^{-1}, 1633 cm^{-1}, 1635 cm^{-1} and in acridine is 1639 cm^{-1}.

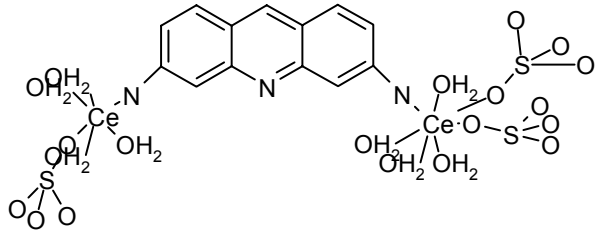
For all four complexes a large band appears in the range of 3300-3400 cm^{-1} that are associated with the presence of coordination water molecules.

Proposed structures for the complexes ***A*** – ***C*** based on analysis are illustrated in figure 1.

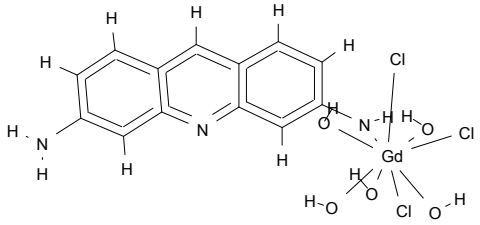
Chemiluminescence experiment results

The method of chemiluminescence is characterized by high sensitivity, a large dynamic range of concentrations of the substances determined, minimum background, no

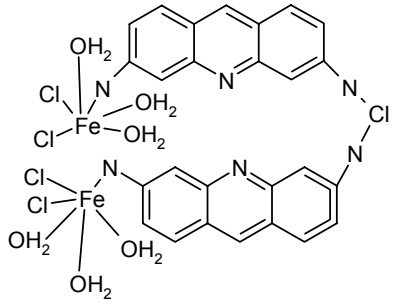
disturbances and light scattering, reproducibility and the possibility of simple and quick analysis [6 - 9].



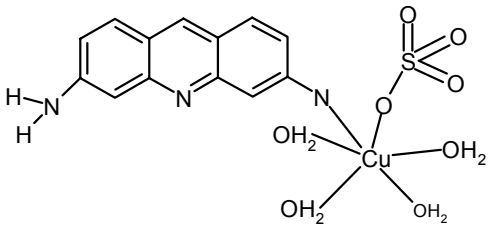
$[Ce_2L_2(SO_4)_3(H_2O)_8]_n$ – A



$[GdLCl_3(H_2O)_5]$ – B



$[\text{-}Fe_2L_2Cl_5(H_2O)_2\text{-}]Cl$ – C



$[CuL(SO_4)(H_2O)]$ – D

Figure 1**. Proposed structures for complexes **A** – **D

For the luminescence studies were recorded the absorption spectra of the complexes in aqueous medium, using Luminol at *p*H = 8.6 (maintained with tris(hidroxymethyl-amino)methane – HCl buffer) and H_2O_2 (10^{-5} mol/L) as reference sample. The absorption spectra are shown in figure 2 and reveal that all the complexes exhibit antioxidant behaviour.

The antioxidant activities of the complexes **A**, **B, C** and **D** are in the range of 50 – 76 %:

$[Ce_2L_2(SO_4)_3(H_2O)_8]_n$ – **A**	75.82 %
$[GdLCl_3(H_2O)_5]$ – **B**	69.90 %
$[Fe_2L_2Cl_5(H_2O)_2]Cl$ – **C**	58.25 %
$[CuL(SO_4)(H_2O)]$ – **D**	50.20%

The luminescence experiments revealed that all the complexes exhibit antioxidant behavior that decreases in the following series:

$$A > B > C > D$$

Antibacterial activity assay

We tested antibacterial activity of substances with the method presented above. The results of measuring minimum inhibition diameter (in mm) for complexes, ligand and salts are presented in table 2.

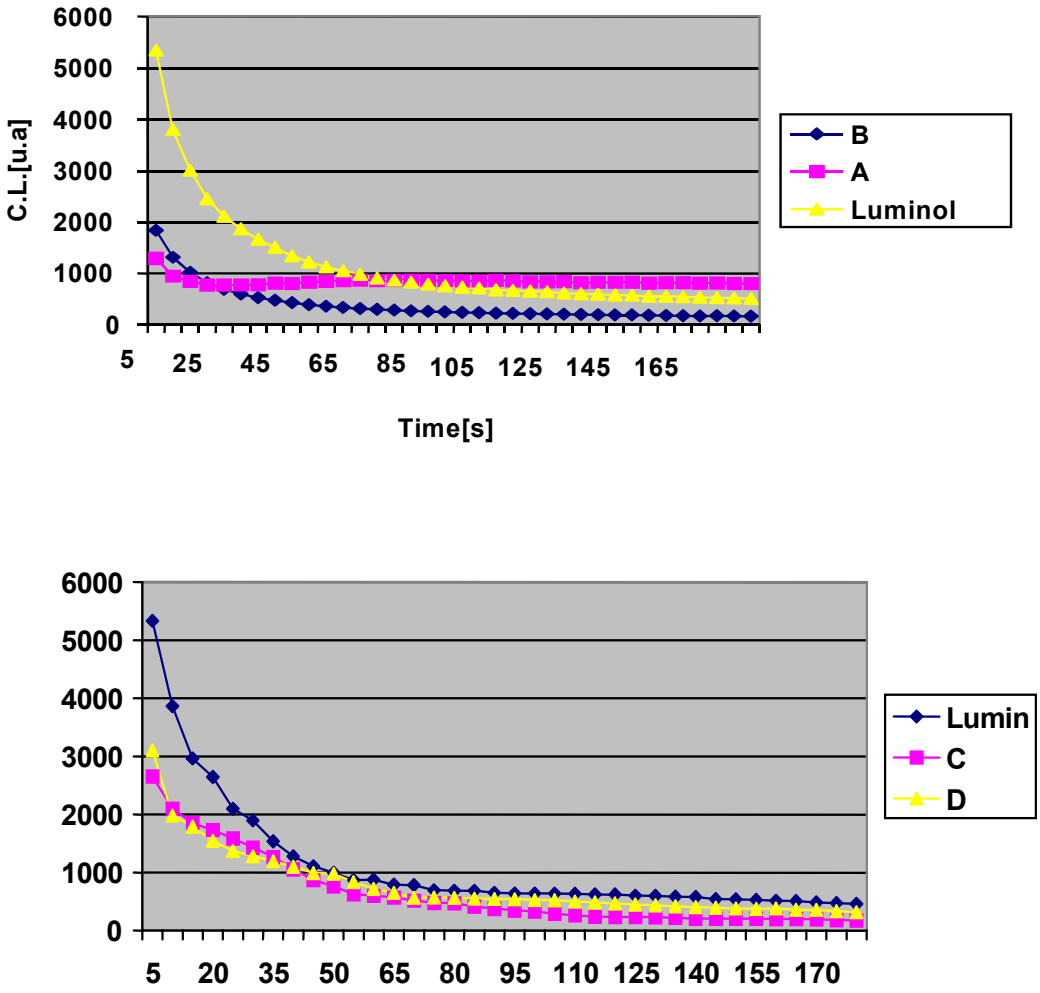


Figure 2. *The chemiluminescence spectra of complexes*

Table 2. *Results of antibacterial activity assay*

Microbial strain		Microbial culture inhibition diameter Ø, [mm]			
		A	B	C	D
Bacilli Gram negative	*Escherichia coli*	1	2	0	0
	Pseudomonas aeruginosa serotip VI	1	3	0	0
	Klebsiella pneumoniae	1	2	0	0
Cocci Gram positive	*Staphyloccocus coagulase positive*	1	5	1	2
	Streptococcus faecalis	1	5	1	2
Fungi	*Candida albicans*	0	4	0	4

CONCLUSIONS

Some new complexes of 3*d* and 4*f* metals with 3,6-diaminoacridine (proflavine, L) were obtained and characterized by elemental chemical analysis, molar electrical conductivity, infrared and chemiluminiscence spectra.

The luminescence experiments revealed that all the complexes exhibit antioxidant behavior, in the following order $[Ce_2L_2(SO_4)_3(H_2O)_8]_n$ > $[GdLCl_3(H_2O)_5]$ > $[Fe_2L_2Cl_5(H_2O)_2]Cl$ > $[CuL(SO_4)(H_2O)]$**.** The values for decay periods suggest that lanthanide complexes may be used as contrast agents for medical resonance imagining (MRI) or luminescent stains for fluoroimmunoassays.

All the complexes have antibacterial action toward Gram positive *Staphyloccocus coagulase positive* and *Streptococcus faecalis*, with an inhibition diameter of 1-5 mm.

The complex with gadolinium presents both antibacterial and antifungic activity, with an inhibition diameter between 2-5 mm, toward all the microbial strain.

The weaker antibacterial action has the iron(III)complex with a maximum 1 mm inhibition diameter over Gram positive stain

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The comparison of antibacterial activity of complexes suggests that 4*f* metal complexes have a stronger action than 3*d* metal complexes.

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