

New Lanthanide Complexes as Potential Fluorescent Labels

OVIDIU OPREA^{1*}, MICHAELA DINA STANESCU², IOANA JITARU¹, LAURA ALEXANDRESCU¹, CRISTINA ILEANA COVALIU¹, LUMINITA CRACIUN¹

¹ University Politehnica of Bucharest, Faculty of Applied Chemistry and Material Science, Department of Inorganic Chemistry, 1-7 Polizu Str., 011061, Bucharest, Romania

² University "Aurel Vlaicu" Arad, 81 Revolutiei Blv., 310130, Arad, Romania

This paper reports the synthesis of two new lanthanum and cerium complexes with general formula $[Ln(H_2O)_2X_3L_2]$ (where $X = NO_3^-$, $Ln = La, Ce$ and $L = 1\text{-phenyl-2-(morpholinyl)ethanone}$), which exhibit fluorescent properties and have potential inhibition activities of monoaminoxidase (MAO) [1-2]. The obtained complexes were characterized by elemental analysis, electronic and IR spectroscopy, molar electric conductivity, as well as thermogravimetric analysis. The chemiluminescent properties of these complex compounds are also investigated.

Keywords: fluorescence, lanthanide complexes, chemiluminescence

Lanthanide complexes have been used for fluorescent labels for a wide variety of analytical methods including high performance liquid chromatography, immunoassay, and fluorescent imaging with time-resolved fluorometric measurements. Introduction of a time-resolved fluorometric measurement permitted the easy distinction of a specific long-lived fluorescence signal from short-lived natural background fluorescence present in most biological samples, reaction wells and optical components. Recently, new fluorescent labels and new instruments have been reported for time resolved fluorescence in environmental analysis as river water, clinical chemistry as allergen assay, and in biochemical analysis as in staining method of proteins on poly-acrylamide gel. The time-resolved fluorometric detection methods using new fluorescent labels improved the detection sensitivity compared to those of previously described methods. Due to their very specific photo-physical and spectral properties, lanthanide complexes are widely applied in various fields, particularly being of major interest for fluorescent applications in biological and clinical assays [3]. The lanthanide complexes have a much longer emission lifetime (hundreds of microseconds) compared to traditional organic fluorescent reagents. The Stokes shifts are very large when the chelates are excited by UV light (310–350 nm) and emit fluorescence in the visible region. The emission profiles are sharp, having a full width at half maximum of only ~10 nm [4].

Our paper presents the synthesis of 1-phenyl-2-(morpholinyl) ethanone (L), a ligand from aminoketone class and its complex compounds with lanthanide ($Ln = La, Ce$). The complex compounds with general formula $[Ln(H_2O)_2X_3L_2]$ ($X = NO_3^-$) have been characterized by chemical analysis, molar conductivity measurements, UV-Vis and FT-IR spectra. The fluorescence spectra as well as quantum yields for both complexes have been also reported. High fluorescence emission intensities can make them useful for analytical applications in biochemistry and biology.

Experimental part

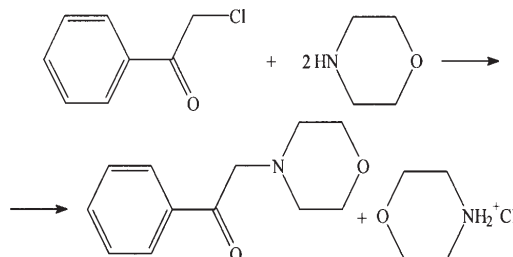
Materials and methods

All reagents and solvents were supplied by Sigma Aldrich.

The synthesis of the ligand

* coviduu73@yahoo.com

The ligand L has been synthesized starting from a mixture of 2-chloro-1-phenylethanone and morpholine in diethylether as solvent. To a mixture of 5.8 mL (66 mmole) piperidine in 20 mL diethylether was added drop wise a solution of 5.1 g (33 mmole) of 2-chloro-1-phenylethanone. The reaction mixture was stirred for 8 h and then left overnight. The white solid of morpholine·HCl formed in time was filtered.



The remaining filtrate was washed with 2x15 mL NaCl solution and dried over $MgSO_4$. The oily product, obtained in 85-90% yields after the solvent evaporation, was purified by distillation. The pure α -aminoketone has the following properties: b.p. = 180 - 181°C; **IR Spectrum** (CCl_4 , cm^{-1}): 680s, 705s, 820m, 860s, 950m, 1010m, 1030m, 1075m, 1117s, 1180m, 1205s, 1230m, 1280m, 1300m, 1350s, 1380s, 1450m, 1550m, 1600s, 1650s, 1680vs, 2600-3030m, 3200-3400w **¹H-NMR Spectrum** ($CHCl_3$, δ ppm): 1.55-2.05 (m, 4H, $H_{3,5}$); 2.85-3.20 (m, 4H, $H_{6,7}$); 4.00 (s, 2H, CH_2); 7.50-8.00 (m, 3H, H_{arom}); 8.00-8.50 (m, 2H, H_{arom}). The experimental data are in agreement with literature [5].

The syntheses of the complex compounds

The complexes have been prepared by refluxing, during two hours, a methanol solution of the ligand L and a metal salt MX_3 ($M = La^{3+}, Ce^{3+}$; $X = NO_3^-$) in a molar ratio 1:1 or 2:1. After the solvent evaporation by heating on a water bath, the separated solid was triturated with cold methanol: diethylether, 1:1 mixture and dried in a desiccator over P_2O_5 . A coloured solid was obtained in yields of 75-80%.

The isolated complexes were characterized by AAS, electronic and IR spectra, as well as differential thermogravimetric analysis.

The metal content of the samples was determined on Pye Unicam atomic absorption spectrophotometer. The

reflectance electronic spectra were recorded on an Able Jasco V560 spectrophotometer.

IR spectra were recorded on a Bruker Tensor 27 spectrophotometer (KBr pellet technique).

NMR-spectrum was performed with a VARIAN A-60 apparatus.

TG-DTA analysis was performed in air in the temperature range 25 - 800°C, at a heating rate of 10°C/min using Paulik Paulik Erdey equipment.

Fluorescence spectra were recorded on an Able Jasco FP 6500.

Molar electrical conductivities have been determined in dimethylformamide solutions at 25°C, with an OK 102/1 RADELKIS Conductometer (measuring range 0.1-0.5 S). Measurements were performed at 25°C in DMF solutions, 10⁻³M.

The antioxidant activities of the two prepared lanthanide complex compounds were investigated in comparison with a chemiluminescent generating system formed by luminol 10⁻⁵ M and H₂O₂ in buffer solution of TRIS-HCl with pH = 8,4 and the final volume was 1 mL. The chemiluminescent signal was registered after 5 s of mixing the system reactants. The obtained results were expressed as relative values of the luminosity intensity taking into consideration the figures of the standards. The antioxidant activity was measured by chemiluminescence method, luminol/H₂O₂ system using Chemiluminometer - TurnerBioSystem (USA) [6]. Results were expressed as activity percents (S%).

Results and discussions

For the ligand **L** a keto-enol transformation is possible. According to molecular mechanics calculations an enol structure seems very plausible, the calculated formation energy for the enol being lower than the value for the keto-isomer **1** (fig.1.). The presence of a weak absorption band at 3200-3400 cm⁻¹ (ν_{OH}) and a strong one at 1680 cm⁻¹ (ν_{CO}) sustains keto-enol equilibrium.

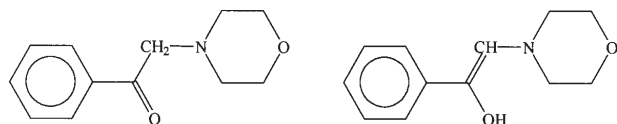
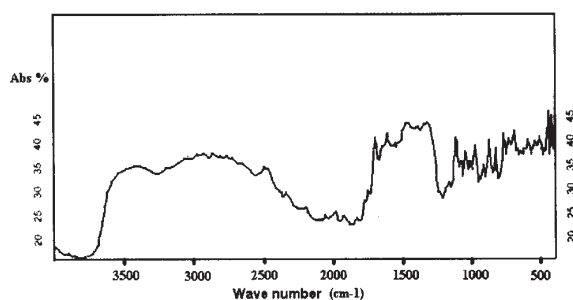
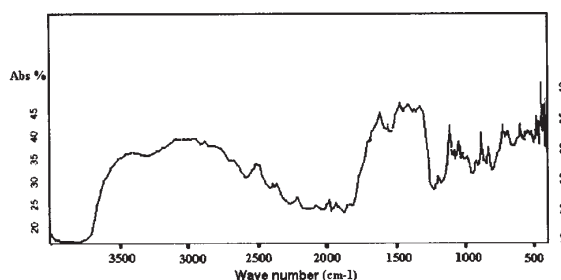


Fig.1. The keto-enol equilibrium



(a)



(b)

Fig.2. The FTIR spectra of [LaL₂(H₂O)₂(NO₃)₃]_n (a) and [CeL₂(H₂O)₂(NO₃)₃]_n (b)

Table 1

MOLAR ELECTRIC CONDUCTIVITY MEASUREMENTS ($\bar{\Lambda}_M$) AND THE ELECTROLYTE TYPE

Complex compound	$\bar{\Lambda}_M$ ($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$)	Electrolyte type
[LaL ₂ (H ₂ O) ₂ (NO ₃) ₃] _n	21	Non-electrolyte
[CeL ₂ (H ₂ O) ₂ (NO ₃) ₃] _n	18	Non-electrolyte

The presence of a hydrogen bond between -OH and N from morpholine nucleus (the calculated N.....O distance 2.80 Å is in agreement with literature data for a hydrogen bond [7]) might be responsible for stabilization of the enol structure. This hydrogen bond might be also responsible for blocking the nitrogen coordinative position (the calculated charges on atoms being O_{OH} -0.303, N -0.025, O_{cycl.} = -0.257), and together with the known oxophilicity of lanthanides leading to the ligand evolving most probably like a bridging, bidentate one.

By comparing the most important bands from the IR spectra of ligand and complex compounds, we were able to formulate the following conclusions (fig.2):

- the presence of the characteristic frequency for the ketone group, ν_{C=O}, in the IR spectra of [LaL₂(H₂O)₂(NO₃)₃]_n (**2**), at lower wave numbers than in the free ligand is an indication of possible coordination of the ligand in ketonic form.

- the absence of the characteristic frequency for the ketone group, ν_{C=O}, in the IR spectra of [CeL₂(H₂O)₂(NO₃)₃]_n (**3**) can be interpreted as a prove that the ligand is coordinated in the enolic form;

- the presence of the characteristic frequency for the OH group (in the region 3200-3400 cm⁻¹) was considered as prove that some water molecules are coordinated;

- the fact that the characteristic bands of the C-N bond are not shifted, and are to be found in complex compounds at 1230 and 1350 cm⁻¹ like in free ligand can be considered as an indication that the N atom is not involved in the coordination process;

- the presence of some modifications in the characteristic frequencies of the ν_{C_{cyc}-O-C} (splitting and shifting towards lower wave numbers) can be attributed to coordination of the heterocyclic oxygen atom;

- the position of the n_{NO} band (from NO₃⁻ ion) in the IR spectra confirms the existence of this anion in the coordination sphere.

The complex compounds are microcrystalline powders, bright red for ([LaL₂(H₂O)₂(NO₃)₃]_n) and orange for ([CeL₂(H₂O)₂(NO₃)₃]_n), soluble in methanol, ethanol, DMF.

The molar electric conductivity measurements established the non-electrolyte nature of these two complexes. In the case of complex [LaL₂(H₂O)₂(NO₃)₃]_n, the bright red colour (fig. 3) can be attributed to charge transfer bands π-d shifted towards visible part of the spectrum.

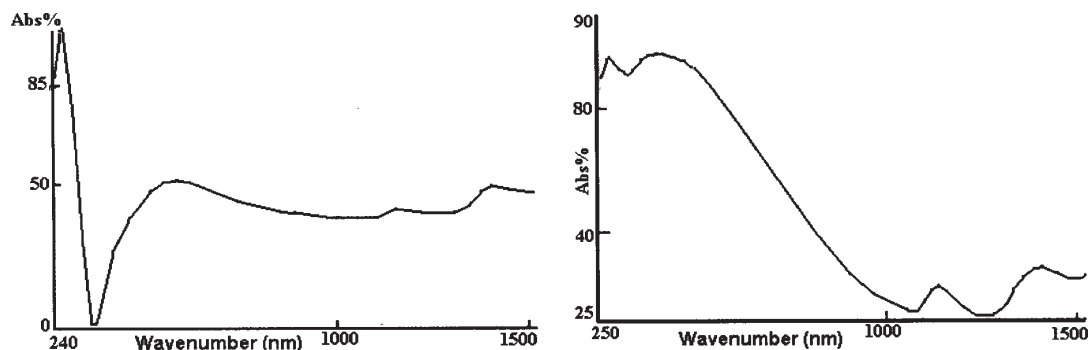


Fig. 3. Electronic spectra of $[\text{LaL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ (a) and $[\text{CeL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ (b)

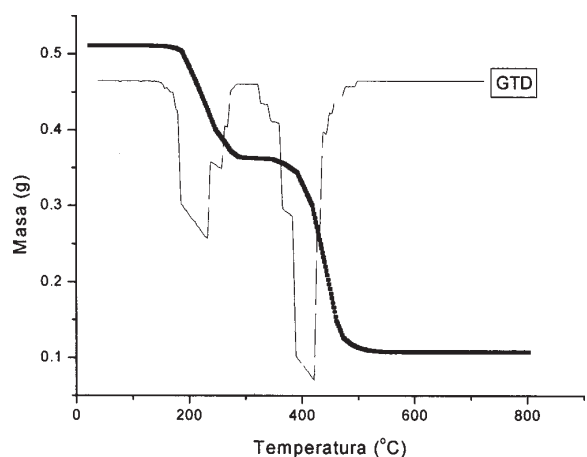


Fig. 4. The TG/DTG curves for $[\text{LaL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$

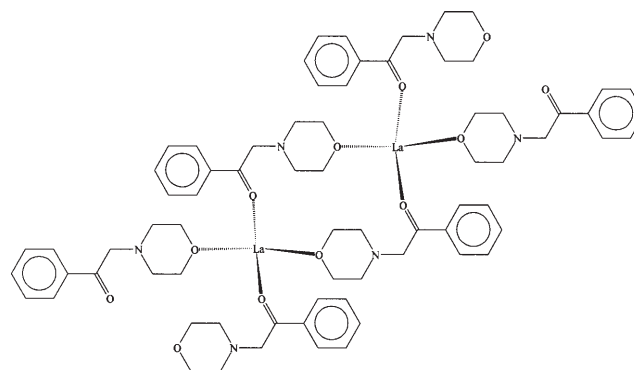


Fig.5. The proposed structure of the $[\text{LaL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$

As it can be observed from the figure 3b, for the cerium complex, the intense charge transfer bands mask the $f-f$ sharp low intensity transitions bands from ground state to the excited state of the $4f^1$ configuration.

The thermal decomposition of complex compounds was studied in order to gather more information about them. The complex compounds present virtually identical patterns of the TG curve, with minor variations in DTG, due to the similar structure. Thermal analysis for the complexes $[\text{LnL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ show a simple decomposition in two steps assigned to: coordination water loss and nitrate

decomposition first step, followed by ligand decomposition in the second step.

The correlation of the obtained experimental data has lead us to propose a polymeric structure as being the most probable (draw omitted the water and nitrate ligands for clarity purposes).

Fluorescence spectra

Fluorescence spectra were recorded for both complex compounds and for ligand as well. The complex compounds exhibit the maximum fluorescence intensity

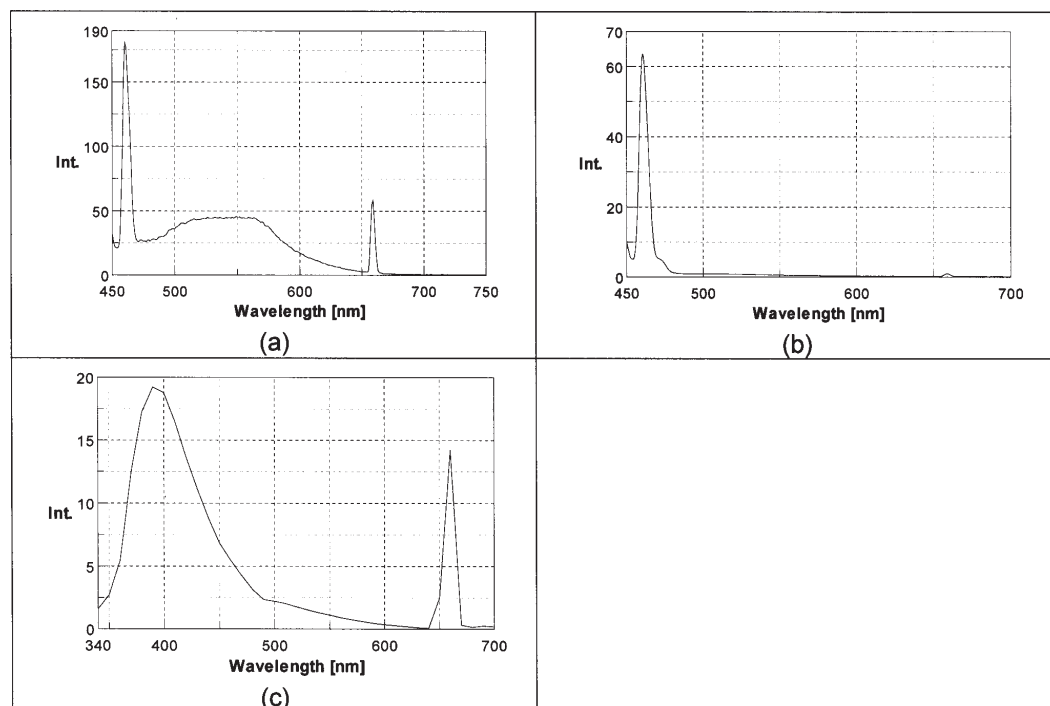


Fig.6. The fluorescence spectra of $[\text{LaL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ (a), $[\text{CeL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ (b) and ligand 1 (c)

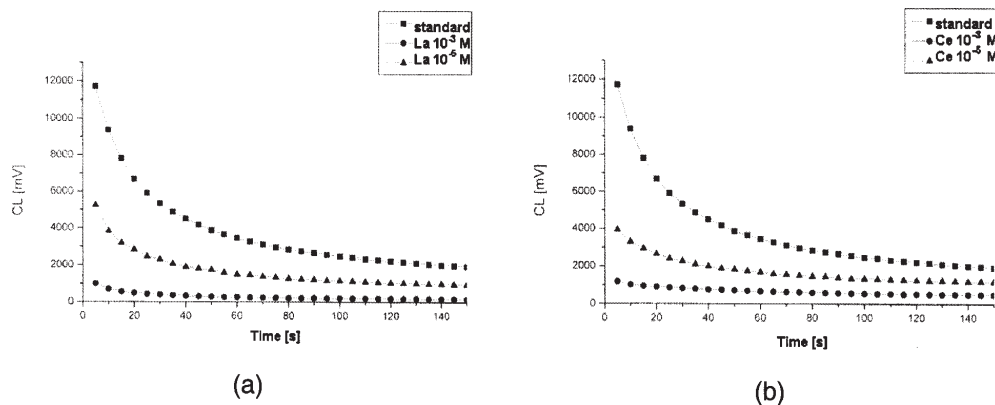


Fig.7. The chemiluminescent signal for $[\text{LaL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ (a) and $[\text{CeL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ (b)

at 460 nm by excitation at 440 nm; while the ligand exhibits the maximum fluorescence intensity at 390 nm by excitation at 330 nm.

The fluorescence spectra of the lanthanum complex (fig.6a) exhibit three distinct emission bands, of different intensities: one very intense band at 460 nm with a sharp profile; a broad band, centred at 544 nm and an intense sharp band at 659 nm. In the case of the cerium complex, following the excitation at 440 nm one can observe only a single sharp band, with lower intensity than in the case of lanthanum complex, at 460 nm (fig.6b). The higher wavelength bands are not present in the cerium complex.

In the case of the ligand, there is one weak, broad and asymmetrical band at 390 nm (fig.6c). Therefore, to conclude, both complexes present significant fluorescence, in the wavelength regions where ligand cannot be made responsible. Even more surprising is the fluorescence of lanthanum complex, knowing the fact that this electronic configuration does not exhibit fluorescence. We assume that the fluorescence emission of the lanthanum complex is a consequence of a nonradiative intramolecular energy transfer, from the excited ligand to the cation, followed by radiative emission from the metallic ion, favoured by the rigid polymeric structure.

Chemiluminescence study

The recording of the chemiluminescent signal decay at 5 seconds interval and the obtained results are expressed relatively to the luminosity of the etalon. We did record the chemiluminescent signal for two concentrations of the complexes, 10^{-5} M and 10^{-3} M.

The lanthanum complex at low concentration (10^{-5} M) has a chemiluminescent signal with about 25% stronger than the cerium complex (fig. 7 a and b), but with a higher rate of decay consequently. At 10^{-3} M the cerium complex has only a marginal stronger chemiluminescent signal.

Conclusions

This paper presents the synthesis and characterization of 1-phenyl-2-(morpholinyl)ethanone ligand and two corresponding lanthanide complexes $[\text{LaL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ and $[\text{CeL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$. Our study shows that complex $[\text{LaL}_2(\text{H}_2\text{O})_2(\text{NO}_3)_3]_n$ exhibits enhanced fluorescence emission, with a very good Stokes shift. The complexity of the molecules and the nature of the emission (i.e., related to complex structure rather than lanthanide / ligand emission) render the structural study of the complexes highly challenging. This fluorescence intensity emission of the lanthanum complex compounds with a suitable ligand could make possible the design of new markers for the study of the inhibitor action mechanism and can be used in the non-invasive medical techniques of visualisation and treatment of some metabolically disorders related to the MAO activity.

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