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ANALYSIS

METAL – LIGAND INTERACTION OF LANTHANIDES WITH COUMARIN DERIVATIVES. PART I. COMPLEXATION OF 3-(1-AMINOETHYLIDENE)-2H-CHROMENE-2,4(3H)-DIONE WITH La(III), Ce(III), Nd(III) AND Ho(III)

MIROSŁAWA ŚWIĄTEK and ALEKSANDER KUFELNICKI

Laboratory of Physical and Biocoordination Chemistry, Medical University of Łódź, Faculty of Pharmacy, 1 Muszyńskiego St., 90-151 Łódź, Poland

Abstract: Solutions of lanthanum(III), cerium(III), neodymium(III) and holmium(III) nitrates with 3-(1-aminoethylidene)-2H-chromene-2,4(3H)-dione (**1**) in 10% v/v dioxane-water medium were used. Coordination modes of **1** with the selected lanthanides have been examined. Hydroxo-complexes with deprotonated water molecules from the inner coordination sphere have been stated in basic medium. Stability constants of the forming complex species were determined by potentiometric titrations using Superquad and Hyperquad2003 programs. The most stable complexes are formed with La(III). The UV-Vis spectra of the Nd(III)-**1** system confirmed the L:M = 1:1 stoichiometry evaluated potentiometrically.

Keywords: lanthanide complex, coumarin derivative, coordination, stability constant

Lanthanides are a group of elements of similar physicochemical properties, which change periodically with the atomic number. Their atom structure differs in the series only in gradual filling of the 4*f* shell. The 4*f* electrons are chemically inert and do not contribute in the bonding, so the chemical reactivity of lanthanides is very similar, as well. The oxidation number is in most cases +3, and the coordination numbers are generally high. The lanthanide ions are larger than the others of the same coordination number (1).

The potentially therapeutical effects of lanthanides has received attention in the end of the 20th century, in spite of their toxicity (2–5). In the center of interest is the anticancer activity of lanthanides and their complexes with coumarin derivatives, which as alone have also antitumor properties (6–8). Recently, a number of new coumarins and their La(III), Ce(III), Nd(III), Sm(III), Gd(III) and Dy(III) complexes had been shown to demonstrate cytotoxic activity on various cancer cell lines, regarding however the toxicity of coumarin towards living organisms (9–17). The antioxidant and anticoagulant effects of coumarins and their complexes with lanthanides are also related to their anticancer properties (18, 19). One of the 4-hydroxycoumarin complexes with Nd(III) showed also an anti-HIV activity (20). Although the complexes were characterized by numerous physicochemical methods – elementary analysis, mass spectral data, IR, ^{1}H-, and ^{13}C-NMR-spectroscopies – there are only few studies regarding the protonation and complexation equilibria that occur in solution (21–23). Moreover, up to now, the fitting procedures common in contemporary coordination chemistry, have not been applied in the lanthanide–coumarin systems.

In the present work, lanthanum(III), cerium(III), neodymium(III) and holmium(III) complexes with coumarin derivative **1** were investigated in mixed solvents. The first three metals were chosen because of their widely characterized biological effects of potential medical application, whereas holmium(III) was selected as one of the heavy lanthanides. Coumarin derivative **1** is a recently synthesized compound that was proved as a ligand in palladium(II) complex, an interesting analogue of carboplatin, of high cytotoxicity towards several cancer cell lines and very low nanomolar range IC_{50} values (24).

* Corresponding author: e-mail: miroslawa.swiatek@umed.lodz.pl

EXPERIMENTAL

Materials

3-(1-Aminoethylidene)-2H-chromene-2,4(3H)-dione (Fig. 1) was synthethized by the group of Prof. Elżbieta Budzisz, Faculty of Pharmacy, Medical University of Łódź (24).

Lanthanum nitrate hexahydrate p.a. (≥ 99.0%) was purchased from Fluka. Cerium(III) nitrate hexahydrate (99.0%), neodymium(III) nitrate hexahydrate (≥ 99.9%) and holmium(III) oxide (≥ 99.9%) were purchased from Aldrich.

Potentiometric titrations

The experiments were performed by means of Molspin Ltd. (Newcastle upon Tyne, England) automatic titration set, a combined OSH 10-10 electrode (METRON Poland) and MOLSPIN.EXE software. The titrations in 10% v/v dioxane-water were carried out in a double-walled thermostated vessel (t = 25°C), starting from pH < 3. The samples of ligand were weighted on a calibrated balance and put into the vessel together with 0.02 mol/L HNO_3 and 2 mol/L KNO_3 solutions, dioxane (0.4 mL) and filled up with water up to total volume of 4 mL. The ionic strength was adjusted to 0.1 (KNO_3) in aqueous phase. The titrant was a carbonate-free standardized 0.1 mol/L NaOH solution (Baker, USA). The total volume of the Hamilton microsyringe in the autoburette (250 μL) was divided into 100 titration points, hence the volume increments of the titrant amounted to 0.0025 mL. In order to attain equilibrium in all the titration points, the default value of maximum drift in delay per 1 s was lowered, varying in particular titrations from the default value 0.09 mV/s down to 0.02 mV/s. On the other hand, the maximum time of waiting for steady readings was enhanced above the default 50 s.

The glass electrode was standardized by using two buffers of known pH: 1) 0.05 mol/L potassium hydrogen phthalate in 0.05 mol/L KNO_3; pH_{25^oC} = 3.926, and 2) 0.01 borax of ionic strength 0.1 (KNO_3); pH_{25^oC} = 9.10 (25). Moreover, the electrode was calibrated in terms of hydrogen ions concentration by titration of a 0.005 mol/L HNO_3 10% v/v dioxane-water solution adjusted to ionic strength 0.1 (KNO_3) with 0.1 mol/L NaOH, temp. 25°C. The values of E_0 and s from equation $E = E_0 - s\,(-\log(H^+))$ were found by Superquad (26, 27) and then used in the input files needed to evaluate the formation constants.

UV-Vis measurements

From among the used lanthanides only Nd(III) was used in the spectrophotometric measurements, since only in this case the wavelength range embraced the accessible UV-Vis region. The UV-Vis absorption spectra of neodymium(III) with **1** were recorded on a Cary 50 Bio spectrophotometer (slit width 1.5 nm, path length 1 cm) at slow scan. The 50% v/v solutions (dioxane-water and also DMSO-water) were prepared in 10 mL flasks using a fixed volume of Nd(III) nitrate water solution, weighted sample of the ligand, 2.0 mol/L KNO_3 to maintain the ionic strength 0.1, 5 mL of dioxane or DMSO and the phthalate buffer. The buffers of various pH (3.9–5.0) were prepared of potassium hydrogen phthalate (Merck), KNO_3, 0.1 mol/L NaOH and water. Total Nd(III) concentration in all the solutions amounted to 1.0×10^{-3} mol/L, whereas the ligand:metal molar ratio ranged from 1:1 to 4:1.

RESULTS AND DISCUSSION

Hydrolysis constants of the lanthanide(III) ions

Hydrolysis constants of lanthanides(III) used had to be determined at first, owing to a lack of such values in the literature for 10% v/v dioxane-water medium. The titrations of samples containing Ln(III) and small excess of HNO_3 at the same conditions of temperature and ionic strength as in the main measurements were carried out repeatedly. The concentration of the ligand amounted $0.75–1.5\times10^{-3}$ mol/L. The number of mmoles of mineral acid added (HNO_3) was equal or higher than that of the ligand. Thus, the titrations with 0.1 mol/L NaOH started from the pH 2.3–2.7. The hydrolysis constants were refined initially by means of Superquad (26, 27) and afterwards by Hyperquad2003 (28), which enables to use more than five individual titrations in the comprehensive file. In the calculations only the acidic part of titration curves could be taken into account because of precipitation of the solid hydrolysis products in more basic solutions. The equilibrium model consisted of three overall formation constants of $LnOH^{2+}$, $Ln(OH)_2^+$ and $Ln(OH)_3$ hydroxo-ions (as the unknown values) and the ionic product of water in 10% v/v water-dioxane solution (determined separately as pK_w = 13.94). The refined constants corresponded to the overall hydrolysis reaction (in simplified notation):

$$Ln^{3+} \rightleftarrows LnH_{-n}^{3-n} + nH^+ \qquad (1)$$

where the cumulative formation constants are defined as:

$$\beta_{10\text{-}n} = (LnH_{-n}^{3-n})\,(H^+)^n\,(Ln^{3+})^{-1},\ n = 1, 2, 3$$

The calculated values of hydrolysis constants are presented in Table 1.

Protonation equilibria

In the molecule of ligand **1** a dissociation of two protons is possible: one of the imine and another of the hydroxyl group. Since both in the Superquad and Hyperquad notations the basic form of the ligand is fully deprotonated ($L = L^{2-}$), the two-step protonation and the corresponding stability constants can be written as follows:

$$L^{2-} + H^+ \rightleftarrows LH^-,\ \beta_{011} = (LH^-)\ (H^+)^{-1}\ (L)^{-1} \qquad (2)$$

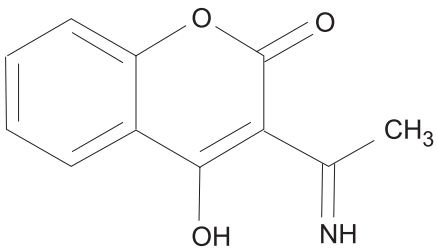

Figure 1. 3-Ethanimidoyl-4-hydroxy-2*H*-chromen-2-one (**1**)

$$LH^- + H^+ \rightleftarrows LH_2,\ \beta_{012} = (LH_2)\ (H^+)^{-1}\ (LH^-)^{-1} \qquad (3)$$

In all the cases calculations confirmed the two assumed protonation constants with mean overall values: $\log \beta_{011} = 4.45$ (8) and $\log \beta_{012} = 7.84$ (18). It follows then that the corresponding stepwise protonation constants are 4.45 and 3.39. These values are in good agreement with the previous data (24) obtained for a 20% v/v dioxane-water solution. An exemplary species distribution diagram is given in Fig. 2.

As can be seen, the neutral and non-coordinating LH_2 species predominates distinctly at pH < 3. Within the range of pH 4.0–4.5, the LH species with deprotonated imine group attains maximum share, whereas the free, fully deprotonated ligand L occurs already above pH > 3.0 and reaches 100% of total concentration at pH > 6.

Complex formation of lanthanide(III) ions with 1

Potentiometric results

In this set of titrations the concentrations of the ligand amounted to 1.5×10^{-3} or 2×10^{-3} mol/L and the

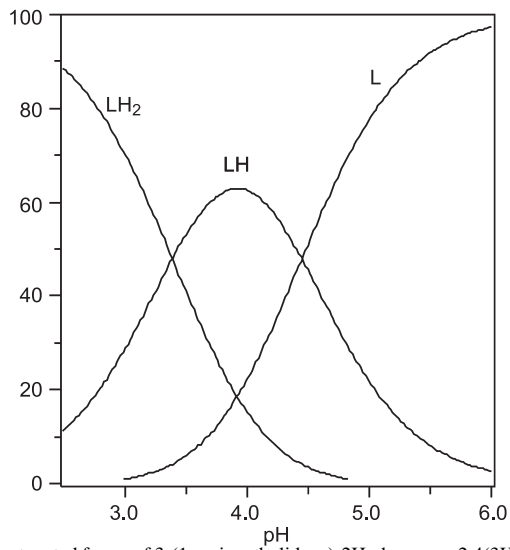

Figure 2. Species distribution of the protonated forms of 3-(1-aminoethylidene)-2H-chromene-2,4(3H)-dione (**1**) in 10% v/v dioxane-water solution. Graphical simulation by HySS2006 (31)

Table 1. Hydrolysis constants of Ln(III) ions in 10% v/v dioxane-water solutions. $I = 0.1$ (KNO_3), temp. 25°C. Comprehensive files consisted of 3–10 individual titrations. Standard deviations in parentheses.

Metal	$\log \beta_{10-1}$	$\log \beta_{10-2}$	$\log \beta_{10-3}$
La(III)	–7.32 (1)	–15.73 (1)	–23.96 (2)
Ce(III)	–6.79 (3)	– [a)]	–21.70 (4)
Nd(III)	–7.62 (2)	– [a)]	–23.73 (2)
Ho(III)	–6.79 (2)	– [a)]	–20.73 (1)

[a)] – Equilibria rejected during refinements.

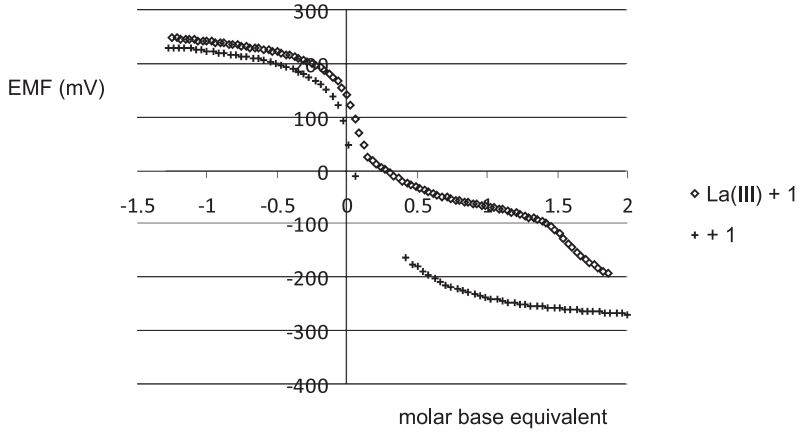

Figure 3. Titration curve of a 4 mL sample containing 0.008 mmole of ligand **1**, 0.004 mmole of $La(NO_3)_3$ and 0.01 mmole of HNO_3 compared with a corresponding titration of the ligand in absence of metal. Titrant: 0.1 mol/L NaOH, solvent: 10% v/v dioxane in H_2O, t = 25°C, I = 0.1 (KNO_3)

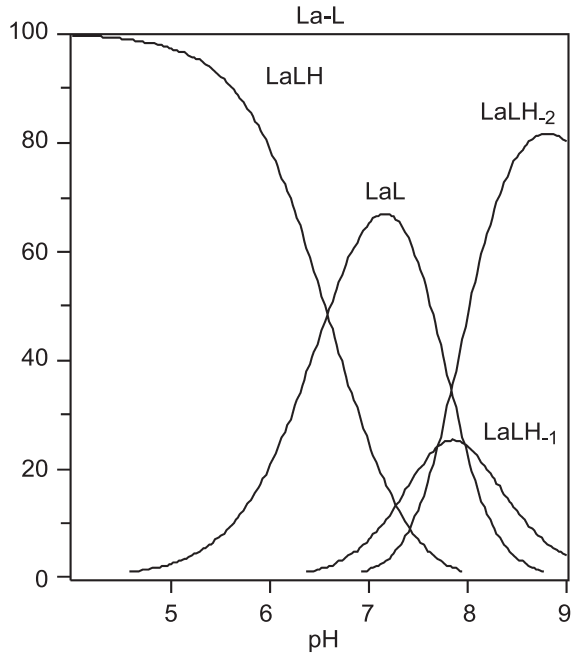

Figure 4. Species distribution of complex species of lanthane(III) with 3-(1-aminoethylidene)-2H-chromene-2,4(3H)-dione in 10% v/v dioxane-water solution. Graphical simulation by HySS2006 (31)

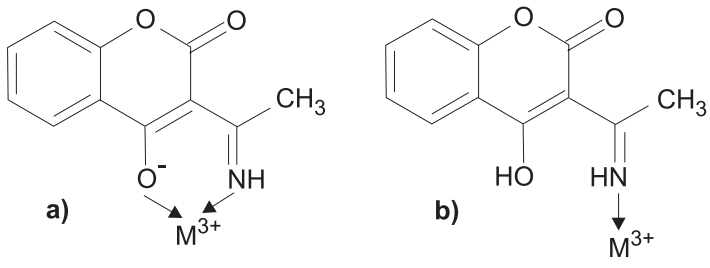

Figure 5. Probable coordination modes of **1** with lanthanides in basic (**a**) and acidic (**b**) solution

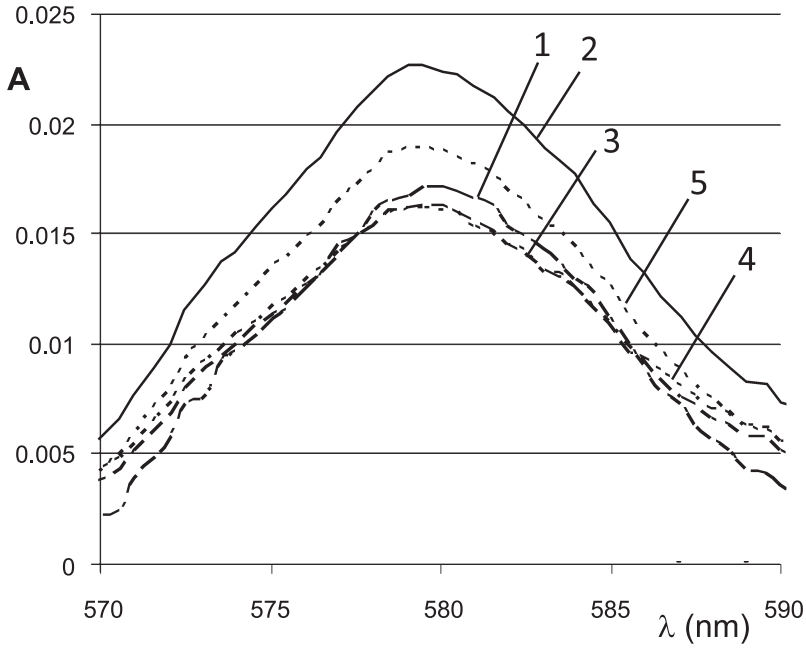

Figure 6. UV-Vis spectra of the ~580 nm band in the Nd(III) – **1** system at various ligand:metal ratio. Solvent: 50% v/v dioxane-water (pH 4.80; phthalate buffer). Total Nd(III) concentration: 1.0×10^{-3} mol/L. Curve 1 – 0:1, 2 – 1:1, 3 – 2:1, 4 – 2.5:1, 5 – 3:1

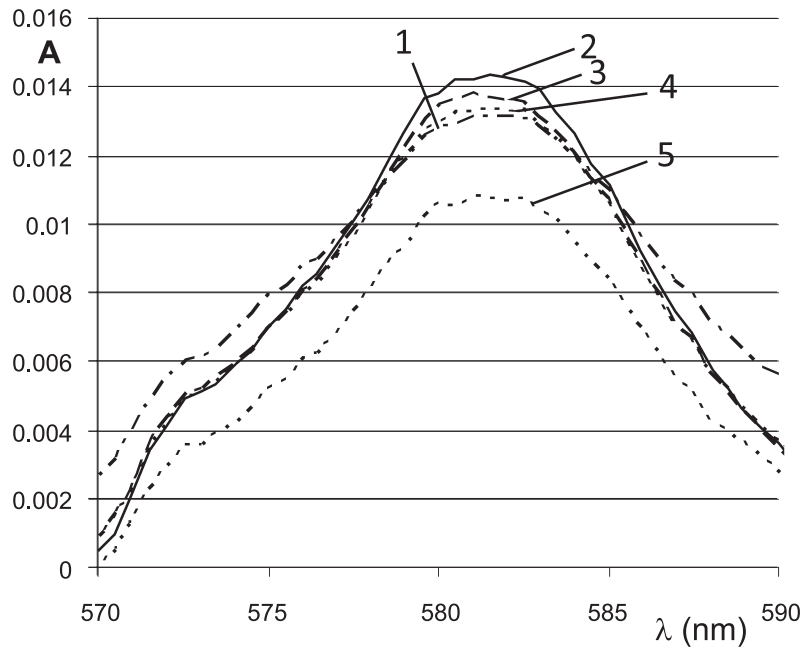

Figure 7. UV-Vis spectra of the ~580 nm band in the Nd(III) – **1** system at various ligand:metal ratio. Solvent: 50% v/v DMSO-water (pH 3.90; phthalate buffer). Total Nd(III) concentration: 1.0×10^{-3} mol/L. Curve 1 – 0:1, 2 – 1:1, 3 – 2:1, 4 – 2.5:1, 5 – 3:1

concentrations of lanthanide(III) nitrate ranged within 2.5×10^{-4}–2.0×10^{-3} mol/L. As a result, ligand-to-metal ratio varied from 8:1 up to 1:1. The mineral acid was added in excess in order to attain pH < 2.8. Although the total quantity of the titrant (0.025 mmole) was sufficient to reach the final pH > 11, the

evaluation of stability constants had to be limited to pH < 8 because of visible perturbations in the titration curves. The probable reasons of such perturbations consisted in precipitation of the final hydrolysis product $Ln(OH)_3$ and the lowered solubility of the ligand in basic medium. The exemplary titration curve in comparison with the titration of ligand in absence of metal is shown in Figure 3. A quite long buffer zone observed in the titration curve during alkalization of the lanthanide-ligand solution (up to molar base equivalent ~1.5) indicates that at higher pH, deprotonated species are formed as a result of evolving protons from water molecules of the inner coordination sphere of Ln(III) ion.

Taking into consideration the presence of two donor groups able to coordinate the metal ion, the following complexes were assumed in the equilibrium model: ML (coordination *via* both the deprotonated groups), MLH (one group coordinating, the second protonated) and two hydroxo species (with participation of one or two hydroxyl groups): MLH_{-1} and MLH_{-2}. The equilibria with the corresponding overall formation constants are as follows:

$$M^{3+} + L^{2-} \rightleftarrows ML^{+},\ b_{110} = (ML^{+})\,(M^{3+})^{-1}\,(L^{2-})^{-1} \quad (4)$$

$$M^{3+} + L^{2-} + H^{+} \rightleftarrows MLH^{2+},\ b_{111} = (MLH^{2+})\,(M^{3+})^{-1}\,(L^{2-})^{-1}\,(H^{+})^{-1} \quad (5)$$

$$M^{3+} + L^{2-} \rightleftarrows MLH_{-1} + H^{+},\ b_{11-1} = (MLH_{-1})\,(H^{+})\,(M^{3+})^{-1}\,(L^{2-})^{-1} \quad (6)$$

$$M^{3+} + L^{2-} \rightleftarrows MLH_{-2}^{-} + 2H^{+},\ b_{11-2} = (MLH_{-2}^{-})\,(H^{+})^{2}\,(M^{3+})^{-1}\,(L^{2-})^{-1} \quad (7)$$

Complexes with two or three molecules of the ligand in the coordination sphere were assumed as well, but in all the cases the corresponding equilibria had been rejected by both Superquad and Hyperquad. The results of refinements are given in Table 2.

Only in case of La(III) a number of titrations could be carried out above pH ~ 9 and by that the constant b_{11-2} could be calculated, as well. The species distribution diagram for the lanthanum(III) system (Fig. 4) demonstrates that complex formation starts from pH ~ 4.5. In acidic medium (pH < 5) MLH is in practice the only complex species. The neutral species ML attains maximum share near to pH ~ 7 and then, subsequent to adding more alkali, the hydrolyzed complex species appear to predominate in solution.

As the molecule of the ligand has two donor groups – hydroxyl and imine – which are deprotonated in basic solution, it may be assumed that in the ML, MLH_{-1} and MLH_{-2} species both the groups participate in coordination of metal ion (Fig. 5a), whereas in acidic medium only one dative bond could be formed, most probably *via* the imine group (Fig. 5b).

Spectrophotometric results

From the UV-Vis spectrum of neodymium(III), the ~580 nm band, corresponding to the hypersensitive transition $^4I_{9/2} \rightarrow {}^4G_{9/2}$ (1, 29) was chosen to observe the changes in absorbance among solutions of various L:M ratio. The lower wavelength part of the spectrum (close to UV) was not suitable because of significant absorption of the NO_3^- anion (in particular the less intensive band of two, near 300 nm, assigned to $n \rightarrow \pi^*$ transition) (30). Hence, the spectra were recorded within the range 570–590 nm. Moreover, the ligand itself does not absorb at this range. In comparison with the potentiometric experiments, the concentration of ligand was the same. Because of low solubility of both ligand and complex, in spite of increased content of the organic solvent (up to 50% v/v), the L:M ratio could not exceed 3:1 for both solvents. Furthermore, the known molar absorption coefficients of Nd(III) at 580 nm are relatively low (~10) (1), which explains the observed low values of absorbance. Thus, the spectrophotometric measurements could serve only as additional confirmation of the potentiometric results.

Indeed, in the spectra with both the organic solvents (Figs. 6 and 7), the maximum of absorbance was attained for ligand-to-metal ratio 1:1, whereas with further increasing the L:M ratio the ~580 nm band gradually diminished. However, this effect was additionally disturbed in the case of dioxane-water solvent. The lower solubility of ligand and complex in 50% v/v dioxane-water seems to be the probable

Table 2. Formation constants of Ln(III) complexes with **1**. Comprehensive files consisted of 3-10 individual titrations. Solvent: 10% v/v dioxane-water. I = 0.1 (KNO_3), temp. 25°C. Standard deviations in parentheses.

Metal	log β_{110}	log β_{111}	log β_{11-1}	log β_{11-2}
La(III)	4.75 (5)	11.74 (6)	–3.70 (10)	–11.16(5)
Ce(III)	3.72 (8)	11.42 (8)	–2.82 (6)	–
Nd(III)	–	9.94 (10)	–5.12 (10)	–
Ho(III)	3.86 (4)	11.07 (2)	– 2.59 (3)	–

reason of absorption enhancement in curve 5 as a result of light scattering (Fig. 6).

CONCLUSIONS

Coumarin **1** is forming mononuclear 1:1 complexes in 10% v/v dioxane-water solution with the selected lanthanides(III): lanthanum(III), cerium(III), neodymium(III) and holmium(III). Both the functional groups participate in coordination of the metal ion. In acidic medium, only one dative bond could be formed, most probably *via* the imine group, whereas in basic medium both the imine nitrogen and hydroxyl oxygen form chelate species. The most stable complexes are formed with La(III), probably due to the highest 4*f* shell spherical symmetry from among the selected metals. The synthesis and characterization of those complexes in solid state seem to be very important in view of its further applications. In particular, by comparison with the recently synthesized Pd(II)-**1** counterpart and also recently synthesized Ln(III) complexes with bis-coumarins, the expectations should be directed towards eventual cytotoxic activity on a variety of cancer cell lines, remembering however the known toxicity of coumarin.

Acknowledgments

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