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Spectrophotometric method for studying the stability of late lanthanide complexes

© Ts.B. Sumyanova, V.R. Prizhilevskaya, N.E. Borisova

Moscow State University, Moscow, Russia
e-mail: ts.sumyanova90@gmail.com

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The results of studying the effect of the central metal ion on the stability of gadolinium and holmium complexes with 1, 10′-phenanthroline-2,9-dicarboxylic acid diamide are presented. The obtained values of the stability constants of the complexes turned out to be an order of magnitude lower than the previously known values for the complexes of these elements with 4,7-dichloro-1, 10′-phenanthroline-2,9-dicarboxylic acid diamide.

Keywords: spectrophotometry, gadolinium, holmium, contrast agents.

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Introduction

Lanthanide complexes are actively used in medicine, especially in cancer diagnostics and therapy. Preparations based on gadolinium complexes [1–4] are used as a contrast agent in medical visualization by magnetic resonance imaging (MRI). Their bactericidal action is also known [5].

The holmium-166 isotope emits high-energy beta particles that are used for therapy, in addition, paramagnetic effects and gamma radiation can be simultaneously used to visualize and control the correct placement of the isotope at the tumor site [6], which allows the use of this isotope for theranostics — simultaneous therapy and diagnosis of the disease.

One of the important characteristics of the complexes that determine their potential application is their stability constant in solution, which indicates the strength of the resulting molecules [7–9]. One of the main methods to determine this constant is the spectrophotometric titration under the condition of absorption by the ligand or complex of light in the UV or visible ranges.

This study is devoted to establishing the composition and stability of lanthanide complexes with 1,10′-phenanthroline-2,9-dicarboxylic acid diamide (Phen). We have previously studied 4,7-dichloro-1,10′-phenanthroline-2,9-dicarboxylic acid diamide (Cl-Phen) [10]. Since the chlorine atom is an electron acceptor, it is assumed that the strength of complexes with the Cl-Phen ligand will be lower than for the unsubstituted analogue (Phen).

1. Research objects and methods

The structures of the studied compounds are shown in Fig. 1 (the synthesis is described in Ref. [11]). Acetonitrile (HPLC−S Gradient Grade, Biosolve BV) was used in the titration. The water content in the solvent is 40 ± 5 ppm and was determined by the Karl Fischer method (C20, coulometric Fischer titrator, Mettler Toledo). We used fluorimetrically pure crystalline hydrates of lanthanide nitrates $Gd(NO_3)_3 \cdot 6H_2O$ and $Ho(NO_3)_{\{3\}} \cdot 5H_2O$ (IREA). The optical absorption was measured on a Hitachi U−1900 spectrophotometer in the range of 200−500 nm with a step of 1 nm at 25 ± 0.5°C in a 10 mm quartz spectrophotometric cell (Hellma).

To determine the time of establishment of chemical equilibrium, solutions of the ligand (Phen), gadolinium and holmium nitrate salts were prepared. The optical density of an equivalent mixture of solutions of the ligand and lanthanide nitrate was recorded for 15 min at the absorption wavelength of the complex (310 nm), using acetonitrile as a reference solution. The time of establishment of equilibrium in the system is defined as the moment after which no further change in optical density is observed.

The analytical absorption wavelength of the complex (λ_{an}) to determine its composition was measured by the Fierordt method as

$$A_x - A_L = f(\lambda),$$

where A_x is the optical density of a mixture of ligand and metal solutions (see above), and A_L is the optical density of a free ligand solution. For λ_{an} we took the value of the

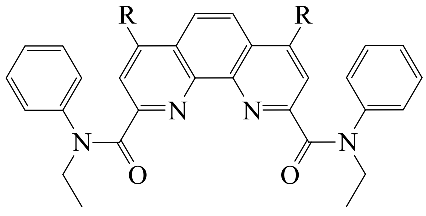

Figure 1. Structure of 4,7-diR-N^2,N^9-diethyl-N^2,N^9-diphenyl-1,10-phenanthroline-2,9-dicarboxamide, where R=Phen, Cl-Phen.

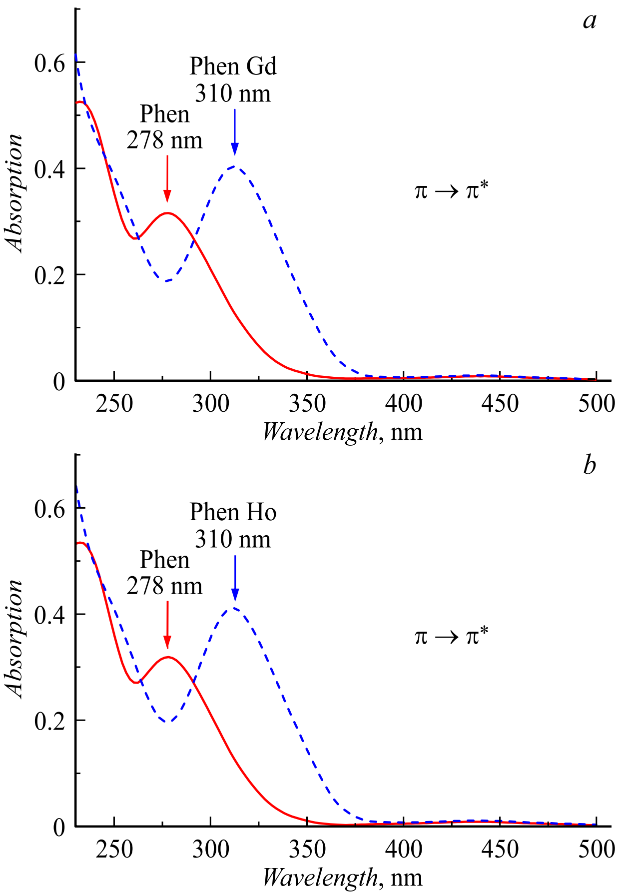

Figure 2. Changes in the absorption spectra of solutions during the formation of the complex, where the solid line denotes the ligand solution, and the dashed line the added excess of the metal nitrate salt, *a* — formation of the gadolinium complex with the ligand, *b* — formation of a complex of holmium with a ligand.

wavelength corresponding to the maximum on the plotted curve $f(\lambda)$.

To determine the composition of the complex, the method of continuous changes (method of isomolar series) [12–14] was used. The method is based on determining the ratio of equimolar solutions of the reactants, which corresponds to the maximum yield of the formed complex compound $\mathrm{Ln}_x\mathrm{L}_y$. If only one complex is formed as a result of the reaction, then the curve of dependence of the complex yield on the composition is characterized by an extreme point, and only one. Such a point corresponds to the maximum possible concentration of the $\mathrm{Ln}_x\mathrm{L}_y$ complex formed by the reaction:

$$x\mathrm{Ln} + y\mathrm{L} \leftrightarrow \mathrm{Ln}_x\mathrm{L}_y,$$

and its position is uniquely related to the stoichiometric coefficients x and y:

$$X = \frac{c_{\mathrm{L}}}{c_{\mathrm{L}} + c_{\mathrm{Ln}}} = \frac{x}{x+y},$$

where c_{Ln} and c_{L} are the initial concentrations of the components.

To do this, we plotted the dependence of $\Delta A = A_{\Sigma} - A_{\mathrm{L}}$ on the concentration ratio at the wavelength λ_{an} and determined the position of the maximum on the curve: $\Delta A = f\left(\frac{c_{\mathrm{L}}}{c_{\mathrm{L}}+c_{\mathrm{Ln}}}\right)$.

To determine the equilibrium constant of the complex formation reaction, a ligand solution ($2\cdot10^{-5}\,\mathrm{M}$) was placed in a cuvette, then aliquots of a metal nitrate solution ($2\cdot10^{-3}\,\mathrm{M}$) in the ligand solution. Absorption spectra were recorded after each addition of a lanthanide nitrate solution until an excess of metal salt of 2 equivalents was reached.

Based on the equations of material balance, the Bouguer–Lambert–Beer law and the law of additivity of optical densities, equations were derived for calculating the equilibrium concentrations of the complex, ligand and metal (the charge of the metal ion is omitted for convenience) ($l = 10\,\mathrm{mm}$) :

The material balance equations are:

$$c_{\mathrm{L}} = [\mathrm{L}] + [\mathrm{LnL}],$$

$$c_{\mathrm{Ln}} = [\mathrm{Ln}] + [\mathrm{LnL}],$$

$$A = A_{\mathrm{L}} + A_{\mathrm{ThL}} = \varepsilon_{\mathrm{L}}1[\mathrm{L}] + \varepsilon_{\mathrm{Th}}1[\mathrm{ThL}],$$

$$[\mathrm{L}] = c_{\mathrm{L}} - [\mathrm{LnL}],$$

$$A = \varepsilon_{\mathrm{L}}1(c_{\mathrm{L}} - [\mathrm{LnL}]) + \varepsilon_{\mathrm{Ln}}\,1[\mathrm{LnL}],$$

$$A - \varepsilon_{\mathrm{L}}1c_{\mathrm{L}} = [\mathrm{LnL}]1(\varepsilon_{\mathrm{LnL}} - \varepsilon_{\mathrm{L}}),$$

$$[\mathrm{LnL}] = \frac{A - \varepsilon_{\mathrm{L}}1c_{\mathrm{L}}}{1(\varepsilon_{\mathrm{LnL}} - \varepsilon_{\mathrm{L}})},$$

$$[\mathrm{L}] = c_{\mathrm{L}} - [\mathrm{LnL}],$$

$$[\mathrm{Ln}] = c_{\mathrm{Ln}} - [\mathrm{LnL}],$$

where $c_{\mathrm{L}}$is the total ligand concentration, c_{Ln} is the total metal concentration in the solution, ε_{L} and $\varepsilon_{\mathrm{LnL}}$ are the molar absorption coefficients of the ligand and the complex, respectively, $A$is the optical density of the solution.

The equilibrium constant K was calculated using the equation $K = [\mathrm{LnL}][\mathrm{L}]^{-1}[\mathrm{Ln}]^{-1}$. The obtained spectrophotometric data were processed using the HypSpec2014 program [15] . This program takes into account the present reagents and equilibrium constants nk. The objective function is given in matrix notation as $U = r^TWr$, where r is the vector of residuals, $r = (y^{obtained} - y^{calculated})$, y are the optical density matrices and W is the weight matrix. To minimize the objective function, the Gauss–Newton–Marquardt method was used, generalized by the system of equations $(J^TWJ + \lambda D)\Delta p = J^TWr$, where J is the Jacobian and Δp is the vector of shifts to be applied to the parameters. D is taken equal to the diagonal elements J^TWr and λ is the Marquardt parameter. The elements of the Jacobian are obtained from the equations of the Bouguer–Lambert–Beer law:

$$A_{\lambda} = l\Sigma_{j=l,na}\varepsilon_{i,j}c_j,$$

$$\frac{\partial A_{\lambda}}{\partial x} = l\Sigma_j\varepsilon_{\lambda,j}\frac{\partial c_j}{\partial x},$$

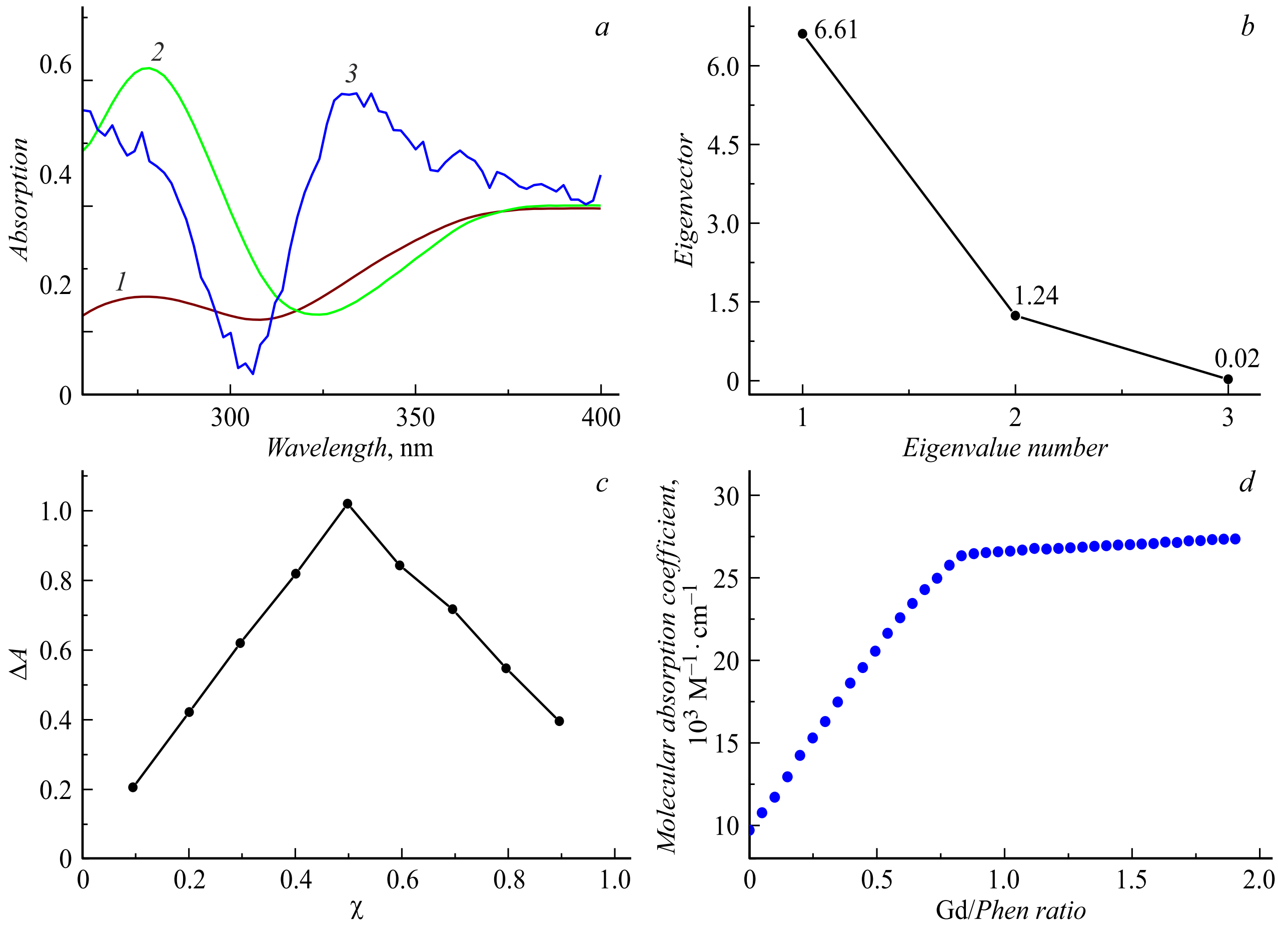

Figure 3. Determining the composition of the complex of phenanthroline ligand with gadolinium nitrate by the method of factor analysis, where *a* are the eigenvectors and *b* are the eigenvalues, as well as the methods of isomolar series *c* and titration curve *d*.

$$\begin{array}{ccc}
0 \cdots 0 & 0 \cdots 0 & 0 \cdots 0 \\
\frac{\partial A_1}{\partial \varepsilon_{1,1}} \cdots \frac{\partial A_1}{\partial \varepsilon_{1,na}} & 0 \cdots 0 & 0 \cdots 0 \\
0 \cdots 0 & \frac{\partial A_2}{\partial \varepsilon_{2,1}} \cdots \frac{\partial A_2}{\partial \varepsilon_{2,na}} & \frac{\partial A_2}{\partial \varepsilon_{2,na}} \cdots 0 \\
\vdots \cdots \vdots & \vdots \cdots \vdots & \vdots \ddots \vdots \\
0 \cdots 0 & 0 \cdots 0 & 0 \cdots \frac{\partial A_{n1}}{\partial \varepsilon_{nl,1}}
\end{array}$$

In addition, the system is subject to the restriction that the material balance equations must be satisfied:

$$T_{\mathrm{Ln}} = [\mathrm{Ln}] + \Sigma_{k=1,nk} p_k \beta_k [\mathrm{Ln}]^{p_k} [\mathrm{L}]^{q_k} [\mathrm{LnL}]^{r_k}$$

$$= [\mathrm{Ln}] + \Sigma p_k c_k.$$

This equation is for lanthanide nitrate Ln, similar equations are derived for L and LnL. The total concentration TLn is obtained based on the initial amount of the substance n_{Ln},

The HypSpec2014 [15] program implements the possibility of performing a factor analysis of the matrix of experimental values of optical densities, which makes it possible to determine the number of independent absorbing components in a solution [16]. Because each absorbing component of the solution has „a unit spectrum“ which is the spectrum for unit concentration and unit optical path length, and this spectrum contributes to the measured spectrum in proportion to the concentration of that component. Eigenvectors are linear combinations of unit spectra of such components, but, unfortunately, the coefficients of linear combinations are unknown, so eigenvectors, strictly speaking, are not absorption spectra of the components. In this case, the number of nonzero eigenvalues will be equal to the number of independent absorbing components of the chemical system.

2. Experimental results

2.1. Absorption spectra

The absorption spectra of solutions of the ligand and its complexes with gadolinium and holmium are shown in Fig. 2. Solutions of the ligand and its complexes with lanthanide nitrates absorb light in the ultraviolet range. The absorption spectrum of the ligand exhibits a maximum at a wavelength of 278 nm, corresponding to $\pi \to n$ transitions of pyridine rings. The addition of a lanthanide nitrate salt to the ligand leads to a bathochromic shift of this band

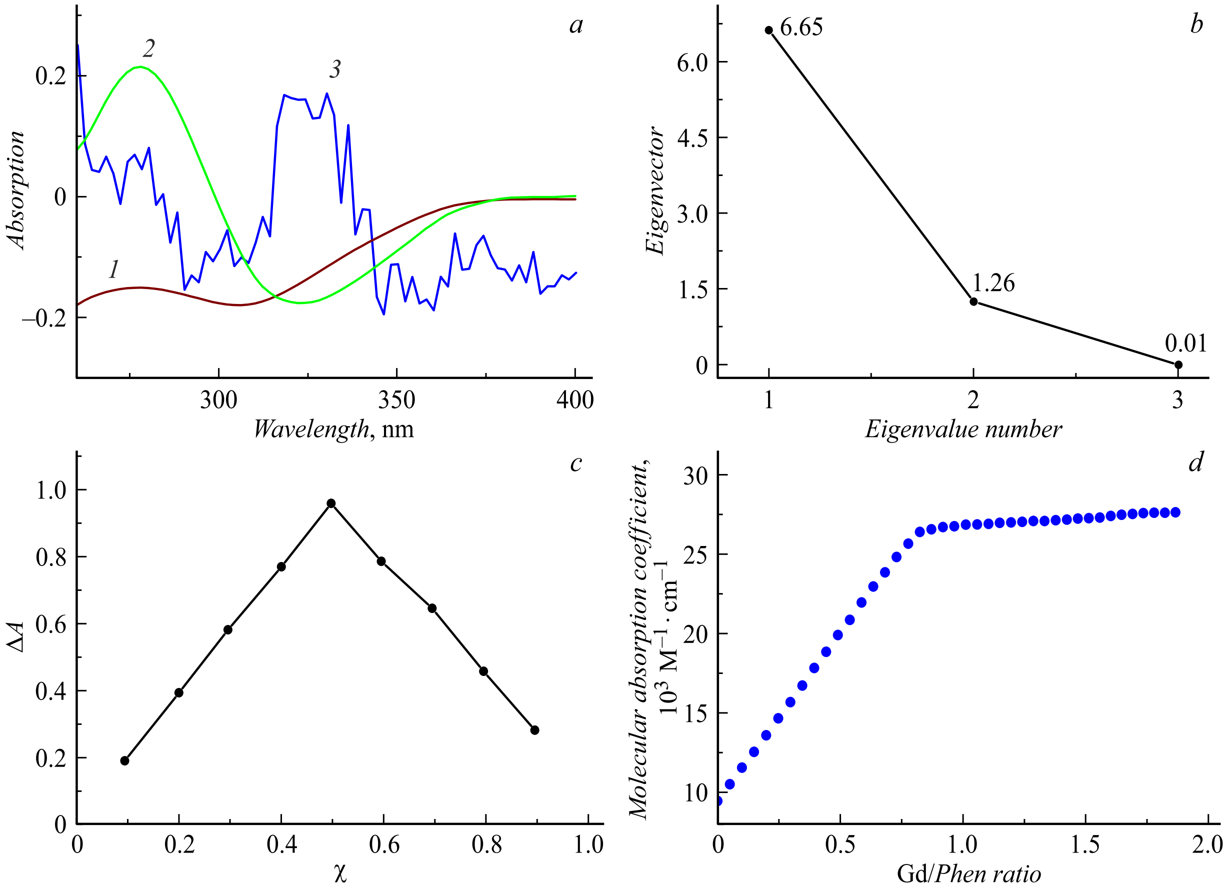

Figure 4. Determining the composition of the complex of phenanthroline ligand with holmium nitrate by methods of factor analysis, where *a*re the eigenvectors and *b* are the eigenvalues, as well as the method of isomolar series *c* and titration curve*d*.

by 32 nm, regardless of the ionic radius of the lanthanide. When conducting a kinetic experiment at a wavelength of 310 nm, it was found that the complex formation reaction proceeds quickly, and absorption stabilizes for 2–4 s, regardless of the metal nitrate salt under study.

2.2. Composition of the complex in solution

The composition of the equilibrium solution was studied using the isomolar series method, the titrimetric method, and factor analysis. An analysis of the optical density matrix for each of the systems under study showed that in both cases the solution contained only three independent absorbing components corresponding to the free ligand, the free metal, and their complex (Fig. 3, *a, b*; 4, *a, b*). Consequently, only one complex particle is formed in the solution. The isomolar series method was used to study the composition of the resulting complexes (Fig. 3, *c*, 4, *c*), which showed that for two ions the composition of the complexes is the same and corresponds to the lanthanide/ligand ratio of 1 : 1. This is additionally confirmed by the titrimetric method (Fig. 3, *d*, 4, *d*): a sharp inflection of the curves is observed at one equivalent of the added metal salt.

2.3. Stability of complexes

The stability constants of complexes of gadolinium and holmium nitrate salts with a ligand (Phen) were determined using spectrophotometric titration (Fig. 5) and processed using the HypSpec2014 [15] program. Figure 6 shows the calculated stability constants of the complexes in comparison with the previously determined constants for the chlorine-containing ligand (Cl-Phen). For these two ligands, an increase in the stability constant of the complexes with a decrease in the ionic radius of the lanthanide is observed. Contrary to our expectations, the stability constants of complexes of lanthanides with an unsubstituted ligand (Phen) are lower than those for a chlorine-containing ligand (Cl-Phen), regardless of the ionic radius of the metal.

Conclusion

The maximum absorption spectra of lanthanide complexes with an unsubstituted ligand (Phen) does not depend on the ionic radius of the metal. It was established that the introduction of two acceptor chlorine atoms into positions 4 and 7 of unsubstituted phenanthroline leads to an increase in the stability of the complexes.

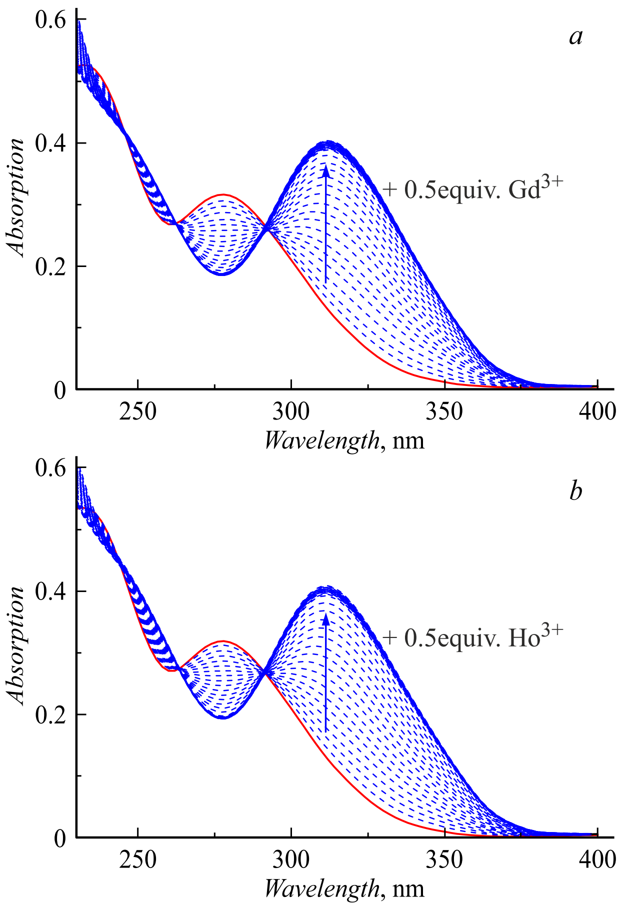

Figure 5. Spectrophotometric data, where 0.5 metal equivalents were added to unsubstituted 1,10′-phenanthroline-carboxamide, and titration was carried out up to 2 equivalents; *a* — gadolinium (III) and *b* — holmium (III) ion.

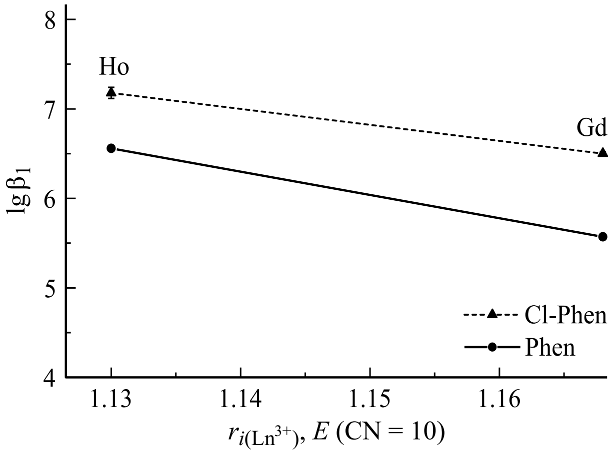

Figure 6. Dependence of the stability constant of complexes on the ionic radius of the metal.

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Conflict of interest

The authors declare that they have no conflict of interest.

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