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Spectral and emission properties of the water-soluble cationic Pd(II) complex with Nile red

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Procedure for the synthesis of a cationic water-soluble Pd(II) complex with 9-diethylamino-5*H*-benzo[*a*]phenoxazin-5-one (Nile red) [PdEnNR]OAc (where En — is ethylenediamine, NR — is deprotonated Nile red, OAc — is outer-sphere acetate-ion) is developed. The positive solvatochromism of the luminescent properties of the Pd(II) complex has been established. The influence of DNA on the spectral-luminescent properties of [PdEnNR]OAc was studied. Intercalation of the complex into DNA in water was found.

Keywords: cyclometallated Pd(II) complex, Nile red, luminescence, intercalation of the Pd(II) complex in DNA.

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Introduction

Phenoxazine dyes effectively absorb light in the visible range, intensely fluoresce in the red spectral area, and since the early 20th century, have found use in histology to detect intracellular lipids [1,2]. Nile red (HNR) — 9-diethylamino-5*H*-benzo[*a*]phenoxazine-5-one (Fig. 1), a benzo-condensed heterocyclic system with a planar structure, reacts with Pt(II) and Pd(II) as a cyclo-metallating ligand to form a family of complexes fluorescing in the red area. Mixed-ligand complexes of platinum metals with Nile red are currently being investigated in several directions: with β-diketonates [3,4] (hereafter [Me(NR)O^O] for use in OLED type LEDs; dimer complexes - as sensors for endogenous CO in living organisms (fish embryos, mice); with polyalkylated Schiff bases — as liquid crystal photoconductors [5–7].

Since the 1960s, there has been a search for platinum metal complexes used as anticancer drugs capable of covalently binding to DNA. Rosenberg discovered the antitumor activity of *cis*-dichlorodiamine Pt(II) (cisplatin) [8]. And from 1979 cisplatin has become an important chemotherapy component for the treatment of certain oncological diseases. Unfortunately, the use of cisplatin is limited by serious dose-limiting side effects and congenital or acquired drug resistance [9].

Since the mid-1970, Lippard and coworkers expanded the field of anticancer drug research into noncovalent interaction of DNA with intercalators — platinum metal complexes with nitrogen-containing ligands (ammonia, ethylenediamine, 2,2′,2′-terpyridine, 1,10-phenanthroline, 2,2′-bipyridyl) [10]. According to their data, cationic Pt(II) complexes with diimine ligands (2,2′,2′-terpyridine, 1,10-phenanthroline, 2,2′-bipyridyl) efficiently intercalate into DNA according to the nearest neighbor exclusion rule: one complex for every two pairs of nitrogenous bases. As an intercalating standard substance for our experiment, we chose the chloride (2,2′-bipyridyl)ethylenediamine Pt(II) (hereafter [PtEnBipy]Cl_2, Fig. 1), whose intercalation has now been proven by a variety of instrumental methods: X-ray diffraction, elastic neutron diffraction, gel electrophoresis, isothermal titrimetric calorimetry, IR linear dichroism, and two-dimensional NMR [11,12].

The relevance of works on the study of intercalator-DNA interactions is of great importance both for the development of theoretical models of biological processes (replication and transcription) and for the development of new antitumor agents. However, the known complexes [Me(NR)O^O] are poorly soluble in water [3,4]. Therefore, we synthesized a new cationic water-soluble palladium (II) — [PdEnNR]OAc complex (En — ethylenediamine, NR — deprotonated Nile red, OAc — outer-sphere acetate-ion) (Fig. 1) to study intercalation. The presence of a positive charge, an aromatic ligand, and the flat-square structure of the complex as recommended by Lippard [10] will favor the interaction of [PdEnNR]OAc with the negatively charged DNA molecule in solution.

Experimental part

9-diethylamino-5*H*-benzo[*a*]phenoxazine-5-one (HNR), palladium acetate (PdOAc), ethylenediamine (En), 2,2′-bipyridyl (Bipy), potassium tetrachloroplatinite ($K_2[PtCl_4]$) glacial acetic acid and calf thymus DNA (Sigma-Aldrich commercial substances, „NevaReactive“) were used without additional purification. All solvents were purified using standard procedures [13].

The cyclometallic [PdEnNR]OAc complex was prepared in several stages according to a procedure similar to the

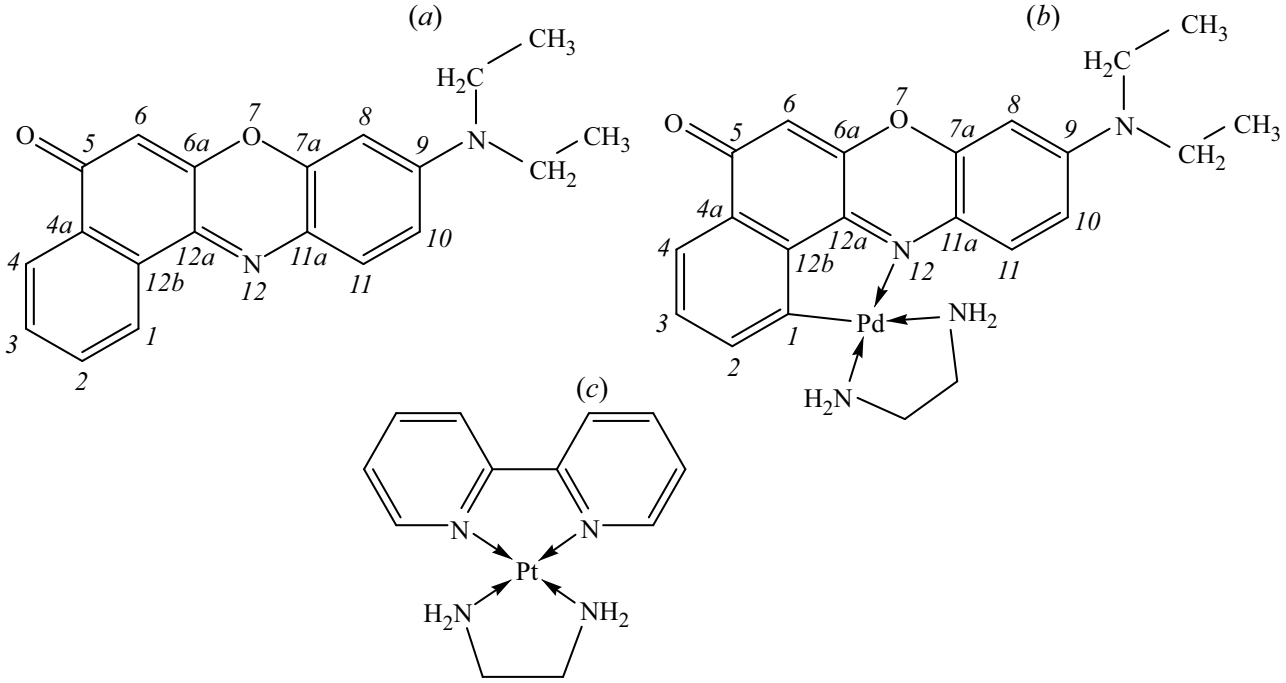

Figure 1. Structural formulas: (*a*) Nile red, (*b*) $[PdEnNR]^+$, (*c*) $[PtEnBipy]^{2+}$.

synthesis of Pd(II) [c]omplexes [14,15]. The difference, as a rule, appears in the color of the resulting substances. First, palladium acetate (21.6 mg, $9.6 \cdot 10^{-5}$ mol) was dissolved in glacial acetic acid (5 ml) by gentle heating. Next, Nile Red (HNR) (29.3 mg, $9.6 \cdot 10^{-5}$ mol) was added to the obtained solution, and the formation of purple color was observed. Then the mixture was boiled for 180 min until it turned a homogeneous dark blue color, after which, it was evaporated to dryness. The obtained precipitate $[Pd(NR)(\mu\text{-}OAc)]_2$ was dissolved in methanol (10 ml), and an aqueous solution of ethylenediamine (0.1 ml, $9.2 \cdot 10^{-5}$ mol) was added, and the color changed to pale blue. The mixture was stirred 30 min at 50°C. After solvent evaporation, the precipitate was dried at 100°C. The weight of the product was 20.8 mg, the yield was 88%. $[PtEnBipy]Cl_2$ complex was obtained according to the method [16].

9-diethylamino-5*H*-benzo[*a*]-phenoxazine-5-one (HNR)

NMR spectrum ^{1}H, δ, ppm: 1.14 t (6H, CH_3, ^3J 7.0 Hz), 3.48 (4H, CH_2, ^3J 7.0 Hz), 6.27 s (1H, H^6), 6.65 d (1H, H^8, ^4J 2.4 Hz), 6.81 d.d. (1H, H^{10}, ^3J 9.1, ^4J 2.4 Hz), 7.60 d (1H, H^{11}, ^3J 9.1 Hz), 7.69 t (1H, H^2, $\langle{}^3J\rangle = 7.4$ Hz), 7.78 t.d. (1H, H^3, $\langle{}^3J\rangle = 7.4$, ^4J 1.0 Hz), 8.10 d (1H, H^1, ^3J 7.4 Hz), 8.53 d (1H, H^4, ^3J 8.0 Hz). The NMR ^{1}H spectroscopy data are in agreement with the reference [17]. NMR spectrum $^{13}C\{^1H\}$, δ, ppm: 12.99 (CH_3), 45.00 (CH_2), 96.55 (C^8), 105.08 (C^6), 110.84 (C^{10}), 123.90 (C^4), 124.77 (C^{11a}), 125.58 (C^1), 130.51 (C^2), 131.50 (C^{11}), 131.60 (C^{12b}), 132.14 (C^3, C^{4a}), 138.80 (C^{12a}), 146.98 (C^{7a}), 151.39 (C^9), 152.40 (C^{6a}), 182.51 (C^5).

Acetate 9-diethylamino-5*H*-benzo[*a*]-phenoxazine-5-onatoethylenediamine palladium (II)[PdEnNR]OAc

NMR spectrum ^{1}H, δ, ppm: 1.15 t (6H, CH_3, ^3J 7.0 Hz), 2.70 br.s (4H, CH_2N), 3.50 q (4H, CH_2, ^3J 7.0 Hz), 4.64 br.s (1H, NH), 5.57 br.s (1H, NH), 6.27 s (1H, H^6), 6.65–6.75 m (2H, H^8, H^{10}), 6.93 ush.s. (1H, NH), 7.05 ush.s (1H, NH), 7.17 d (1H, H^{11}, ^3J 8.8 Hz), 7.32 d (1H, H^2, ^3J 7.4 Hz), 7.39 t (1H, H^3, $\langle{}^3J\rangle$7.6 Hz), 7.65 d (1H, H^4, ^3J 7.5 Hz).

NMR spectrum $^{13}C\{^1H\}$, δ, ppm: 13.01 (CH_3), 45.09 (CH_2), 44.60, 46.62 (NCH_2CH_2N), 97.65 (C^8), 106.80 (C^6), 110.27 (C^{10}), 121.30 (C^4), 122.84 (C^{11a}), 128.00 (C^{11}), 130.20 (C^3), 131.26 (C^{4a}), 136.05 (C^2), 144.18 (C^{12b}), 148.18 (C^{7a}), 150.48 (C^{12a}), 150.97 (C^9), 152.06 (C^{6a}), 153.64 (C^1), 184.15 (C^5).

IR spectrum (KBr), ν, cm^{-1}: 3309 (N-H), 1580, 1407, 1348, 1290, (C=C, C=N), 1637 (C=O).

Absorption spectrum in ethanol, λ_{max}, nm, (extinction coefficient, $l \cdot mol^{-1}cm^{-1}$): 263 (13200), 299 (4050), 330 (4000), 443 (1800), 623 (13550).

Found, %: C 53.17; H 5.13; N 10.37. $C_{24}H_{28}N_4O_4Pd$. Calculated, %: C 53.89, H 5.24; N 10.48.

Spectral studies were performed using the equipment of the Center of Collective Use „Physical and Chemical Methods for Research of Nitro Compounds, Coordinated,

Biologically Active Substances and Nanostructured Materials" of the Inter-Disciplinary Resource Center of Collective Use „Modern Physical and Chemical Methods of Formation and Research of Materials for the Needs of Industry, Science and Education" of Herzen State Pedagogical University of Russia. NMR spectra ^{1}H, ^{13}C^{1}H, ^{1}H–^{1}H dqf-COSY, ^{1}H–^{1}H NOESY, ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC were registered on Jeol ECX400A spectrometer with operating frequencies of 399.78 (^{1}H) and 100.53 (^{13}C); solvent — DMSO-d_6. Residual solvent signals were used as an internal standard. The proton signals were interpreted by the splitting nature (spin-spin coupling constants (SSCC)). Spin-spin coupling between protons was detected by the corresponding cross-peaks in the spectra ^{1}H–^{1}H COSY. Experiments H^8, H^{10} were used to reliably attribute protons ^{1}H–^{1}H NOESY, namely the presence of corresponding cross peaks with methylene group protons NCH_2 due to the Overhauser nuclear effect. The signals of protonated carbon atoms in the ^{13}C NMR spectra were reliably related by the corresponding cross-peaks in the ^{1}H–^{13}C HMQC spectra, and the signals of quaternary (non-protonated) carbon atoms — by cross-peak analysis (hetero-nuclear SSCC through 2–3 bonds) in the spectra ^{1}H–^{13}C HMBC.

IR spectra were obtained on Shimadzu IR-Prestige-21 Fourier spectrometer in KBr pellets. Elemental analysis was performed on a EuroVector EA3000 (CHN Dual) analyzer. Electronic absorption spectra — on SF-2000 („OKB Spektr", St. Petersburg, Russia). Luminescence studies were carried out at room temperature on Fluorat-02-Panorama spectrofluorimeter (GC „Lumex", St. Petersburg, Russia).

The titration of $[PtEnBipy]Cl_2$ and [PdEnNR]OAc complexes with aqueous DNA solution consisted of recording the absorption spectra of Pt(II) or Pd(II) complex solutions (volume of complex solution — 2.5 ml, concentration — 10^{-5} mol·l^{-1}) while sequentially adding 10 μl of DNA solution (3.2 mg DNA dissolved in 5 ml water).

Findings and discussion

[PdEnNR]OAc complex was synthesized according to the following equations:

1) $PdOAc_2 + 2AcOH = H_2[PdOAc_4]$,
2) $2H_2[PdOAc_4] + 2HNR = [Pd(NR)(\mu\text{-}OAc)]_2 + 2AcOH$,
3) $[PdNR(\mu\text{-}OAc)]_2 + 2En = 2[PdEnNR]OAc$.

The composition and structure of [PdEnNR]OAc were confirmed by IR, NMR ^{1}H and ^{13}C{^{1}H} spectroscopy involving homonuclear (^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY) and heteronuclear (^{1}H–^{13}C HMQC, ^{1}H–^{13}C HMBC) experiments, and in comparison to the uncoordinated HNR data.

Table 1 shows the coordination-induced shift ($CIS = \delta_{complex} - \delta_{ligand}$), which was calculated as the difference between the chemical shifts of the carbon atoms of the complex ($\delta_{complex}$) and uncoordinated ligand — HNR (δ_{ligand}). The data show that the carbon atoms closer to the palladium atom (C^1, C^2, C^{12a}, C^{12b}) have a high CIS value, indicating a decrease in electron density at the atom in question. The largest CIS is the carbon atom C^1, which has been deprotonated and formed a chemical bond with palladium.

Table 1. Coordination-induced shifts (CIS, ppm) of carbon

Carbon	C^1	C^2	C^3	C^4	C^{4a}	C^5	C^6	C^{6a}
CIS	28.06	5.54	−1.94	−2.60	−0.88	1.64	1.72	−0.34
Carbon	C^{7a}	C^8	C^9	C^{10}	C^{11}	C^{11a}	C^{12a}	C^{12b}
CIS	1.20	1.10	−0.42	−0.57	−3.50	−1.93	11.68	12.58

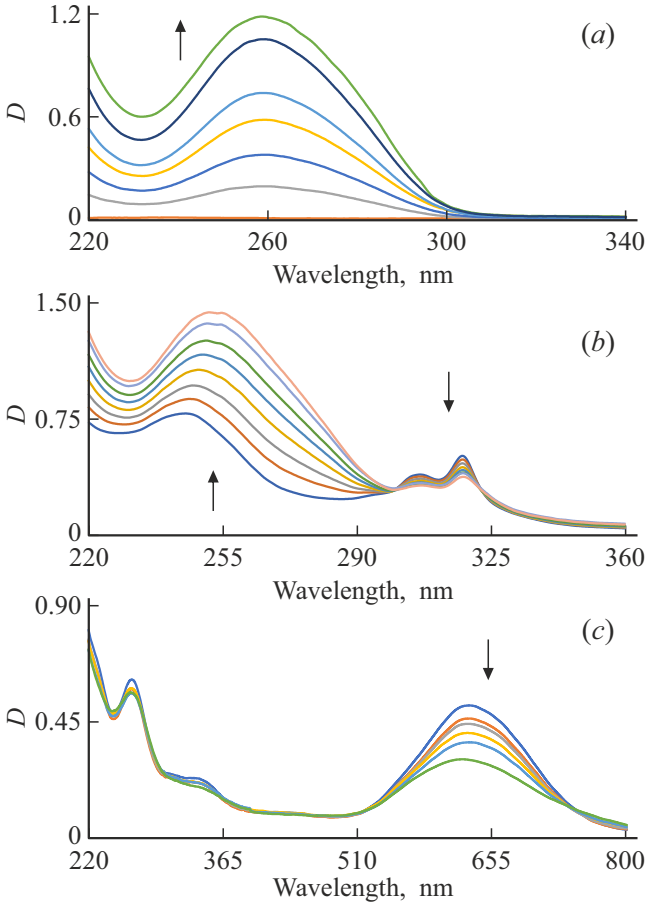

Figure 2. Changes in the absorption spectra of aqueous solutions with increasing DNA concentration: (*a*) water, (*b*) $[PtEnBipy]Cl_2$, (*c*) [PdEnNR]OAc. The arrows indicate the direction in which the spectra change with increasing DNA concentration.

The electronic absorption and emission properties of the HNR compound and the complex will be considered within the framework of the theory of localized molecular orbitals [18]. It is known [19,20] that HNR has positive solvato-chromism, which manifests itself in a batochromic shift of absorption and fluorescence maxima when moving from nonpolar solvent to polar solvent (Table 2). The increase in the dipole moment of the HNR molecule during the light quantum absorption and transition to the excited

Table 2. Luminescence (nm) maxima of HNR and $[PdEnNR]^+$ in some solvents (in parentheses —solvent polarity, kcal/mol [13], n/r — insoluble)

Complex	Benzene (54.0)	DCM (64.2)	Acetone (65.7)	DMFA (68.5)	DMSO (71.1)	Ethanol (81.2)	Methanol (83.6)	Water (94.6)
HNR	576	603	613	626	634	639	638	insoluble
$[PdEnNR]^+$	insoluble	675	680	687.5	697.5	700	703.5	719

Note. DCM dichloromethane, DMFA dimethylformamide, DMSO dimethyl sulfoxide

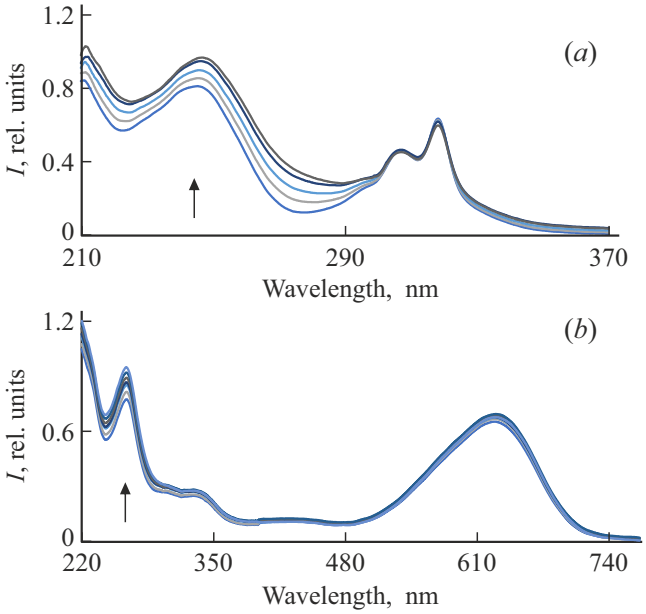

Figure 3. Changes in the absorption spectra of water-ethanol solutions with increasing DNA concentration: (*a*) $[PtEnBipy]Cl_2$, (*b*) [PdEnNR]OAc. The arrows indicate the direction in which the spectra change with increasing DNA concentration.

state is due to intramolecular charge transfer from the amino diethyl group to the carbonyl group: the molecule excitation is accompanied by rotation of the amino diethyl group and departure from the chromophore plane. Positive solvato-chromism is also characteristic of [PdEnNR]OAc (Table 2). Comparing the spectral luminescence properties of the uncoordinated ligand (HNR) and the known complexes [Me(NR)O^O] [3,4] with those of the new complex $[PdEnNR]^+$, we can conclude that they are affected by both the nature of the metal and the donor-acceptor characteristics of the other ligand.

HNR absorption spectra are characterized by a number of spin-resolved intra-ligand transitions of different intensities. The position of the long-wave band depends on the solvent polarity, and in complexes — on the nature of the metal and the other ligand. This is due to the charge-transfer nature of this transition and the partial admixture of the electron density of the metal complexer (Pt(II) or Pd(II)) [3,4]. When studying the intercalation of the [PdEnNR]OAc complex into DNA, we found a decrease in the optical density of the long-wavelength part of the spectrum, which is responsible for the introduction of this part of the molecule into the DNA helix.

Comparison of the changes in the absorption spectra (Fig. 2) of our chosen standard $[PtEnBipy]Cl_2$ and the new complex — [PdEnNR]OAc caused by titration with DNA solution reveals several similar elements. First: an increase in the concentration of DNA in the solution leads to an increase in the optical density of the solution in the absorption area of the nitrogenous DNA bases (260 nm). Second, the absorbance of the intra-ligand-type long-wavelength transition localized at Bipy and NR decreases. Third, isosbestic points appear in the absorption spectra of the complexes, indicating one product of the reaction — an intercalate consisting of DNA and the Pt(II) or Pd(II) complex cations embedded in it. However, after all the cations of the solution complexes are embedded in the DNA, another addition of the DNA solution leads to the violation of the isosbestic points.

Replacing water with 65%-water ethanol (Fig. 3) resulted in partial reproduction of the previously observed effects in the absorption spectra: an increase in the optical density of the solutions only in the absorption area of DNA nitrogenous bases ($\sim$ 260 nm). The long-wave absorption bands (for $[PtEnBipy]Cl_2$ 305−318 nm, for [PdEnNR]OAc 622 nm) did not change their optical density with increasing DNA concentration. This is due to the fact that in water, the intercalation of the complex particle into DNA is partially screened by the aromatic heterocyclic ligand (Bipy or NR), while in ethanol solution, this does not occur.

When [PdEnNR]OAc aqueous solution was titrated with DNA solution, a decrease in fluorescence intensity similar to the absorption spectrum of the complex was observed in the fluorescence spectrum (Fig. 4). Changing the solvent (water to 65% ethanol solution) did not change the complex fluorescence spectra.

Thus, obtaining a water-soluble cationic complex [PdEnNR]OAc made it possible to study its intercalation (intermolecular interaction with DNA) by spectral-luminescent methods. The presence of the planar aromatic ligand Nile red in [PdEnNR]OAc and bipyridyl in $[PtEnBipy]Cl_2$ promotes the incorporation of complexes between adjacent nitrogenous base pairs of the DNA double helix in water, thereby conditioning stacking by $\pi-\pi$-interaction.

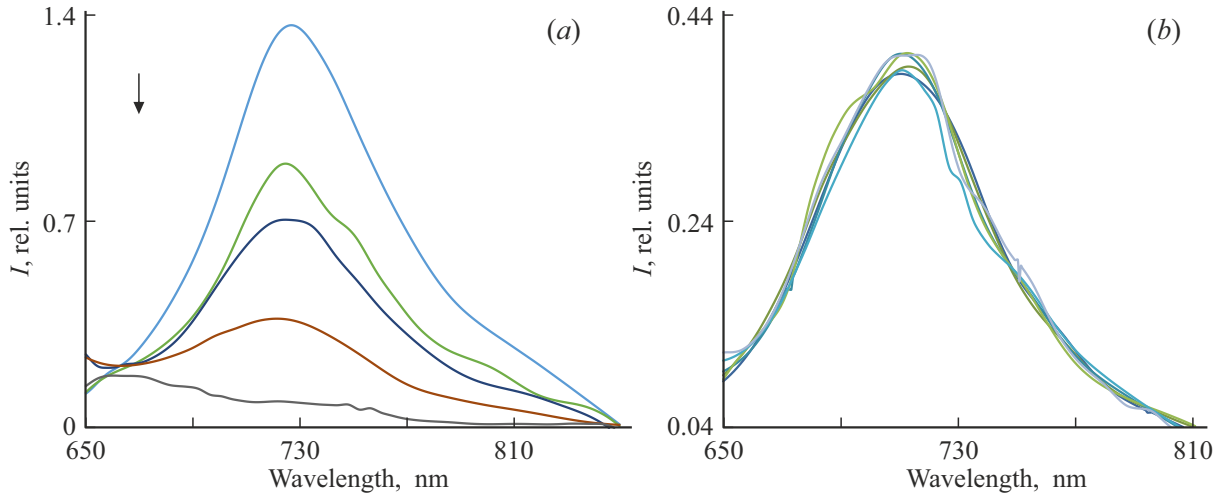

Figure 4. Changes in the luminescence spectra of the [PdEnNR]OAc complex with increasing DNA concentration: *a* — in water, *b* — in 65% ethanol solution.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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