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Photolysis and Quantum-Chemical Calculations of the Nalidixic Acid Radical States

A. V. Polischuk[a,b], **T. B. Emelina**[a], **O. Cramariuc**[c],
V. I. Chukharev[c], **T. E. Karaseva**[a], **and V. E. Karasev**[a]

[a] *Institute of Chemistry, Far Eastern Branch, Russian Academy of Sciences,*
pr. 100-letiya Vladivostoka 159, Vladivostok, 690022 Russia
e-mail: emelina@ich.dvo.ru

[b] *University of Turku, Turku, Finland*

[c] *University of Technology, Tampere, Finland*

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Abstract—The nalidixic acid transient spectra were analyzed and the scheme of photo-conversion of the acid was suggested. At a large delay of laser pulses the transient absorption spectra contain the bands due to the dissociation of the products of the cation- and anion-radical photodecomposition in the micro- and millisecond range. By quantum-chemical calculations we revealed that at the transition to the ion-radical state a proton migration occurs subsequent to the movement of the center of gravity of the negative charge. The phototransformation of nalidixic was found to proceed with a TICT-effect.

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The antibiotics of quinolone class (fqH) have found wide application in medicine. However, the tendency of some antibiotics to phototoxicity [1] requires the study of the influence of light on the composition and phototransformations of the short-living intermediates. This paper presents the results of laser flash photolysis and quantum chemical calculations of the anionic, neutral and cationic protolytic forms of nalidixic acid (nlqH). The numbering of atoms in the structure of nlqH is shown in Fig. 1.

The primary photochemical processes at the interaction of photons with the compounds of fluoroquinolones is intra- or intermolecular electron transfer between the fragments with the transition of nalidixic acid into the singlet S_1-excited state.

The stepwise photophysical and photochemical processes within nanosecond and microsecond ranges are accompanied by competing processes that include energy migration, nonradiative energy loss, electron and proton transport, and luminescence. The primary photochemical process in the case of nalidixic acid is commonly regarded as the intramolecular electron transfer.

We believe that the processes of protonation or deprotonation of quinolones occurring at the intra- or intermolecular electron transfer in the course of the photoexcitation play a key role in the acid–base catalysis of antibiotics. It was of interest to follow the spectral-kinetic and photochemical properties of nalidixic acid in acidic and basic environments for the cationic, neutral and anionic forms. In the study of photolysis of nalidixic acid we found the qualitative

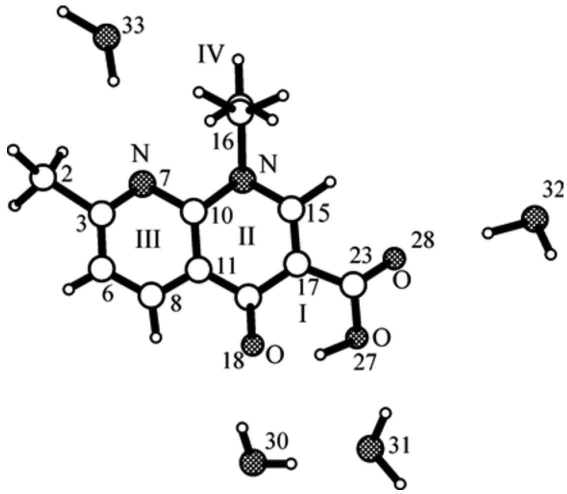


Fig. 1. Structure of nalidixic acid with numbering of its atoms (Arabic numerals) and fragments (Roman numerals).

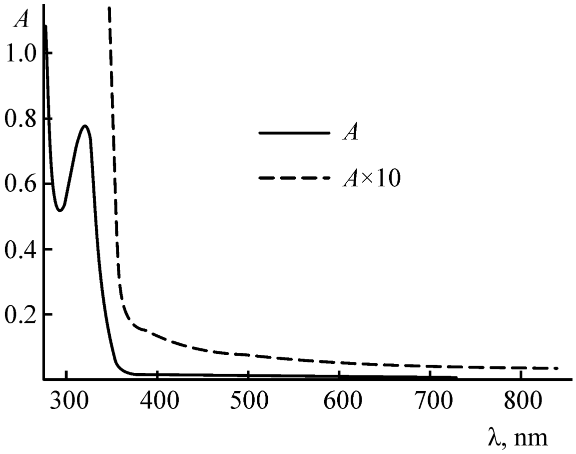


Fig. 2. Absorption spectrum of nlq^- in water, pH = 6.0, c = 1×10^{-4} M.

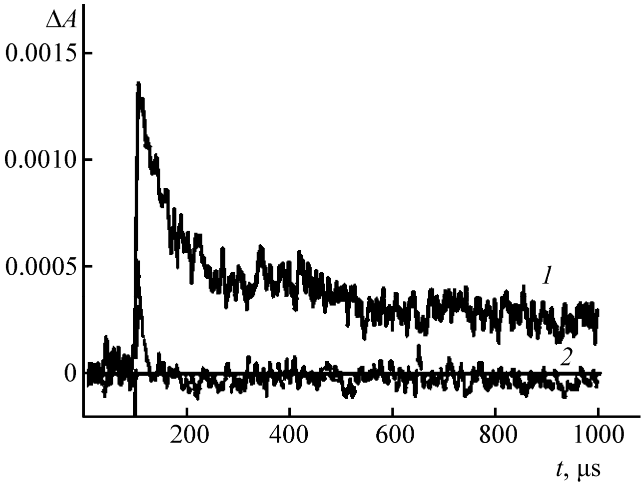


Fig. 3. Examples of kinetic curves of the laser flash photolysis (λ = 355 nm) at the selected wavelengths (*1*) 680 nm and (*2*) 420 nm.

differences in the recorded absorption spectra of excited electronic states.

The laser flash photolysis of fluoroquinolones when several bands have been detected in the visible and near infrared region of the transient spectra is described in a number of papers [2–4]. The bands assignment is usually related to the anion- and cation-radical states of the norfloxacin, ciprofloxacin, ofloxacin, enoxacin, and moxifloxacin [3, 4]. There is no consensus on these problems, because the charge (electron) transfer in the primary photoreaction can occur both in a singlet and a triplet excited states (see schemes 1–3). For example, in [5] the quantum yield of singlet and triplet states of ofloxacin was quantitatively estimated and it was shown that 20% of the molecules decomposed into a radical-cation and a solvated electron: $^1(\text{ofqH})^* \rightarrow \text{ofqH}^{\bullet+} + e_{aq}$. In turn, at the interaction of the hydrated electron with ofqH in the ground state an anionic form $\text{ofqH}^{\bullet-}$ is produced. Both the position and the magnitude of the signal generated by the ion-radicals depends on the composition and concentration of the buffer. It was found in [3] that the bands A and B with λ_{max} 610 and 700 nm respectively in the transient spectra of norfloxacin, ciprofloxacin, and enoxacin respond differently to the composition and concentration of phosphate and acetate buffer solution. The quenching rate of the signal with λ_{max} = 610 nm assigned to the triplet-triplet absorption $^3A^*$ increases in the presence of phosphate buffer from 2.3×10^8 M^{-1} s^{-1} to 5.6×10^8 M^{-1} s^{-1} with an increase in pH from 5.8 to 9.0. However, no such effect was observed in acetate buffer. In turn, the band at λ_{max} = 700 nm attributed to the triplet excited state $^3B^*$ is quenched by molecular oxygen at a rate of 2.6×10^9 M^{-1} s^{-1}. The lifetime of the triplet state $^3A^*$ according to [3] lies in the nanosecond range, while the lifetime of $^3B^*$ is a little less than 2 μs.

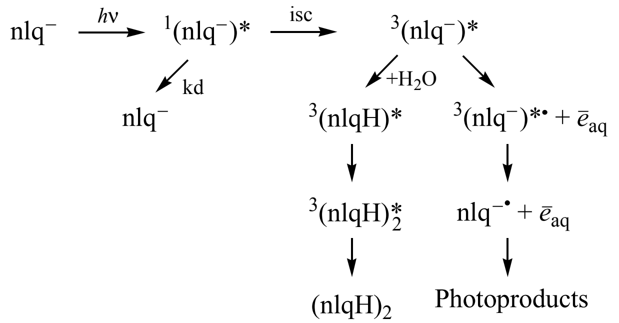
Using the method of nanosecond kinetic spectroscopy we studied the dynamics of transient absorption spectra of aqueous solutions of nalidixic acid at pH 6.0.

Figure 2 shows the absorption spectrum of nlq^- in water at pH 6.0. Figure 3 shows the kinetic curves at two selected wavelengths indicating that at least two exponents should be used for the approximation. The amplitude spectra of the components of double-exponential global approximation with the characteristic time 49 and 890 μs are shown in Fig. 4. More exponents in the approximation do not improve significantly the root-mean-square deviation.

The first time peak at 49 μs is characteristic of the excited triplet-triplet state of nalidixic acid. The second peak we assign to the radical state of the photo-product (Scheme 1). The absorption band in the transient spectrum at 700 nm with a characteristic time 890 μs can be attributed to the long-living radical states $nlq^{\bullet-}$. The conceptual scheme of primary photo-conversions of the nalidixic acid anionic form can be represented as shown in Scheme 1.

According to Scheme 1 after the initiation nlq^- can return to the ground state by nonradiative energy loss, or transform in the triplet excited state (isc, intercombination conversion). There are two possible

Scheme 1.



ways of photo-conversion of the latter. The first way consists in the interaction of nlq$^-$ with the proton of water and its transition into a neutral form capable in the future of excimerization and dimerization [6]. The second way leads to the loss of an electron and the formation of a radical from of nlq$^-$, followed by the cleavage of $^\bullet$COOH group and the formation of the photo-product.

Analyzing the transient spectra of the triplet-triplet absorption of a protein complex with nlq$^-$, Monti et al. [4] indicate the formation of a radical pair TyrO$^\bullet$–nlq$^{\bullet 2-}$ (TyrO is tyrosine). The absorption band at 650 nm is assigned in [4] to the radical state of the anion form nlq$^{\bullet 2-}$, and 780 nm band, to the radical form of tyrosine, which has transferred its electron to nalidixic acid at the formation of the complex compound. This is consistent with our assignment of the band at 620 nm with lifetime 49 μs to ^{3}(nlq$^{-\bullet}$)*, the anion-radical triplet state. This assignment is consistent with the values of the nalidixic acid lifetime (up to 100 μs) estimated from the phosphorescence spectra of nlqH in different buffer solutions [4].

For the neutral form of nalidixic acid, taking into account the quantum-chemical calculations, the scheme of photoconversions is possible (Scheme 2).

In turn, $^\bullet$COOH + H_2O → HCOOH + OH$^\bullet$.

According to calculations, the ionization of the neutral molecule of nalidixic acid (Scheme 2) leads to a decrease in the electron density mainly on the atoms O^{18} (0.43 e) and O^{27} (0.1 e). The numbering of atoms is shown in Fig. 1. The transition of the electron density is followed by the intramolecular proton transfer from atom O^{27} to atom O^{28}. At the transition of the radical (nalH)$^\bullet$ into the excited state (nlqH)$^\bullet$* due to the transfer of electron density from O^{28} to O^{18}, the transition of the negative charge is followed by the proton migration, first to the O^{27} atom and then to the atom O^{18} (Scheme 2).

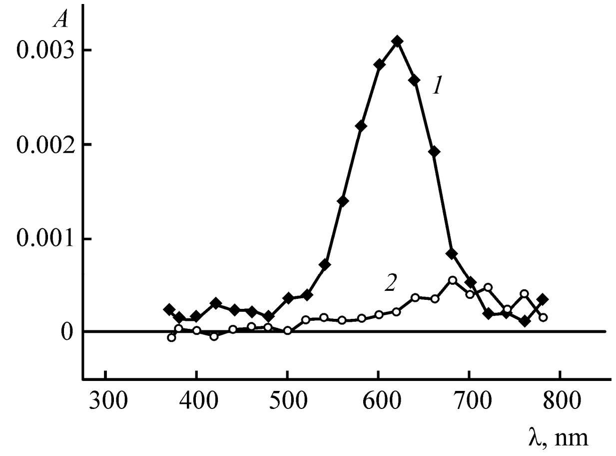
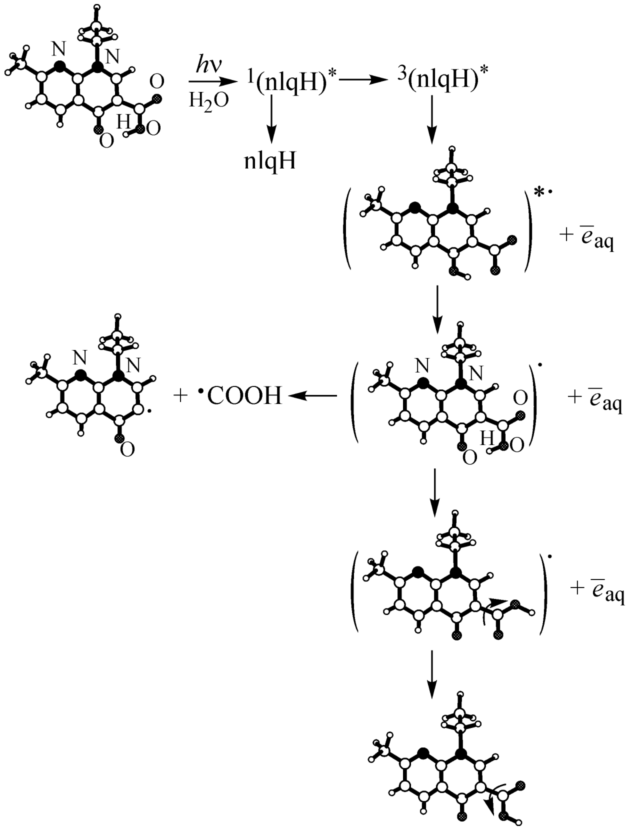


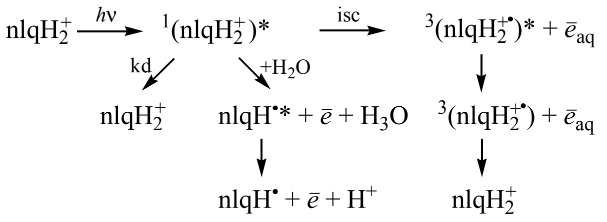
Fig. 4. Transient absorption spectra of nlq$^-$ ($c = 2\times10^{-5}$ M) with characteristic time (*1*) 49 μs and (*2*) 890 μs.

The decay of this excited state proceeds with a stepwise jump of proton from the atom O^{18} to O^{28}. Thus, the effect of intramolecular charge transfer with *twisting* is observed (twisted intramolecular charge transfer, TICT). Such a restructuring of the molecules is the limiting step of the decay of the excited radical

Scheme 2.



Scheme 3.



state. This fact explains the increase in the lifetime of excited radicals $(nalH)^{\bullet}*$.

The photo-transformation of the cationic form $nlqH_2^+$ proceeds according to Scheme 3.

As can be seen from Schemes 1–3, the process of the formation of ion-radical states includes a cleavage of electron, which in aqueous medium undergoes hydration. According to [4, 5], the absorption band of the hydrated electron falls to the region of 720 nm. This approach is consistent with the results of [2], where from the data of ^1H NMR spectroscopy the quantum yield of the photodecomposition of nalidixic acid in methanol with the formation of photo-product of the formic acid, formaldehyde, and trace amounts of hydrogen peroxide was estimated. In the presence of dissolved oxygen the quantum yield of the nalidixic acid photolysis may vary. Since the molecule of nlq^- is surrounded by the hydrate shell, the electron transfer can be accomplished from the water molecule forming a hydrogen bond with the oxygen atom of carbonyl group. The presence of a hydrated electron in the fluoroquinolone aqueous solutions was noted in several studies [3, 4]. The redox reactions and the reactions of protonation of quinolones are an important aspect when discussing the possible participation of fluoroquinolones in redox catalysis with the participation of DNA gyrase.

Table 1. Energy dfference between the highest occupied and lowest unoccupied orbitals (eV) in the different protolytic forms of nalidixic acid in the gas phase and surrounded by water molecules[a]

Structure	S_0	D	Q
nlq^-	10.20	8.70	3.50
nlqH	10.80	8.68	3.31
$nlqH_2^+$	10.88	9.39	3.26
$nlq^-\cdot 4H_2O$	10.34	6.53	2.45
$nlqH\cdot 4H_2O$	10.89	7.35	3.46
$nlqH_2^+\cdot 4H_2O$	10.99	6.62	2.86

[a] S_0 is ground state, D is doublet-radical state, Q is quartet-radical state.

According to quantum-chemical calculations, in the gas phase at the excitation of the radical forms of nalidixic acid a part of the electron density is fairly evenly redistributed from the fragment III (Fig. 1) on the atoms of the ring II and the pseudoaromatic ring I. A similar process is observed also when the solvent is taken into account.

Stability of the radical states follows also from the fairly large energy distances between the HOMO and LUMO of nalidixic acid in different protolytic forms (Table 1). However, at the formation of an adduct with water molecules through hydrogen bonds the charge transfer leads to a significant decrease in the energy gap between HOMO and LUMO of the radical forms of nalidixic acid in the ground and excited states (Table 1), as seen from the red shift of the bands in the absorption spectra of the excited states.

Comparing the total energies of compounds nlqH and $nlqH\cdot 4H_2O$ suggests that the presence of water molecules stabilizes the complex by 83.11 J mol^{-1}, which corresponds to the sum of the energies of formation of hydrogen bonds. The same occurs in the ion-radical states of the other protolytic forms of the nalidixic acid. The interaction between oxygen atoms of the carbonyl and carboxy groups of nalidixic acid with water results in an additional polarization of water molecules. This results in the difference of the charges of two protons of the same H_2O molecule by up to 0.1 e, thereby increasing the ionic component of the bond between nlqH and H_2O. The distances between the oxygen atoms and two hydrogen atoms are also different, the difference is up to 0.03 Å. The transfer of the electron density from the hydrogen atom leads to a slight weakening of the bond of oxygen with the hydrogen atom and increases the O–H distance. At the transition of the radical state of the cationic form of nalidixic acid to an excited state one of the hydrogen atoms is removed to form H_3O^+ cation and a neutral form of nlqH.

All the above said clearly indicates that water molecules contribute to the charge transfer in the ion-radical states of nalidixic acid.

An important characteristic of the reactivity of the radical complexes is the distribution of free valence indices F_A reflecting the chemical properties of the

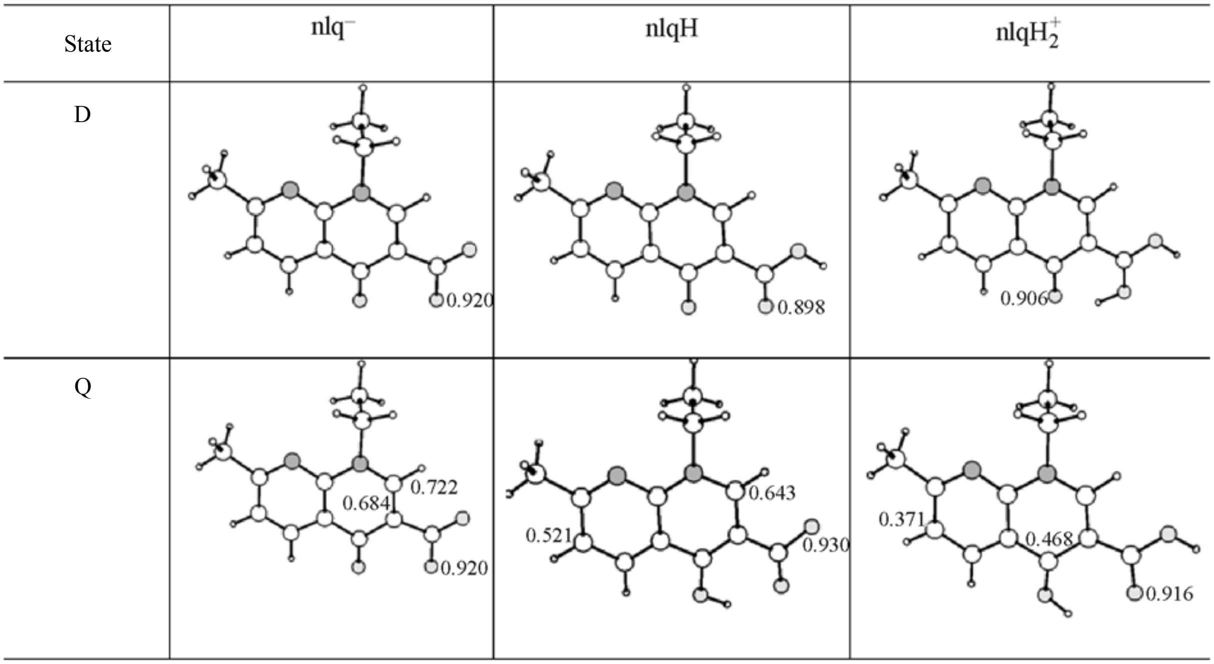


Fig. 5. Distribution of free valence indices over the atoms in the different protolytic forms of nalidixic acid.

molecules. The atoms with the maximum value of free valence are the most reactive (Fig. 5).

A consequence of the intramolecular charge transfer is the change in the bond orders within the molecule and in the free valence indices. Figure 5 shows the distribution of free valence indices of various forms of nalidixic acid in the gas phase in the doublet-radical (D) and quartet-radical (Q) states. According to calculations, the maximum index of free valence in the radical state $(nalH)^{\bullet}$ corresponds to O^{18}, and in the $(nalH)^{\bullet *}$ state, to the atom O^{28}. Thus, the transition of the radical into the excited state changes the location of the reaction centers of the molecules. When a water molecule appears in the first coordination sphere of nalidixic acid, the oxygen atoms with maximum values of the F_A, as expected, are blocked by water molecules, and the carbon atoms become the reaction centers. At the transition of radicals to the excited state the location of the reaction centers of molecules is changed, and therefore a different reactivity and different ways of forming new chemical bonds by the radicals in the ground and in the excited states is presumable.

Moreover, the process of radicalization of the anionic and neutral forms of nalidixic acid is accompanied by the distortion of the planar structure of complexes: the COO^- group rotates around the C–C bond (the so-called, TICT-effect) and the O^{18} atom is deviated from the plane of the quinoline rings. The energy barrier to the internal rotation in the gas phase is 0.37 eV, and in water, 0.07 eV, thus, in this case, water molecules catalyze the TICT effect.

The translation of the radicals of both protolytic forms of nalidixic acid into the excited state leads to the changes in the bond orders between atoms and to a decrease in the dipole moments. The bond order of

Table 2. Bond orders of the C^{17}–C^{23} bonds in the different protolytic forms of nalidixic acid in the gas phase and accounting for the solvent

Bond order	State[a]	nlq^-	nlqH	$nlqH_2^+$
Gas phase	S_0	0.865	1.012	1.018
	D	1.028	0.946	1.149
	Q	1.031	0.990	1.201
Accounting for water molecules	S_0	0.904	0.996	1.005
	D	0.815	0.978	0.948
	Q	0.826	1.024	0.982

[a] S_0 is ground state, D is doublet-radical state, Q is quartet-radical state.

C^{17}–COOH bond at the transition to a radical state decreases, and at the transition to an excited radical state, increases. Thus, at the radicalization of the complex increases the relative mobility of the COOH fragment, and at the transition of the radical into the excited state this bond becomes more rigid (Table 2).

Table 2 shows that the bond order of the quinoline ring with the COO group is mostly less than unity, indicating disappearance of the π-component of this bond [7]. The disappearance of the "flattening" factor like the π-conjugation also indicates the formation of the TICT-state.

Thus, the transient spectra of nalidixic acid have individual character with respect to both the position and the intensity of individual components, and to the characteristic lifetime of the triplet-triplet transitions and the relatively long-living radical products. For first time it was found that in the transient absorption spectra the bands appear which we attribute to the dissociation of the cation- and anion-radical products of photodecomposition of the nalidixic acid hydrate. The quenching of the signals of ion radicals proceeds in the μs and ms ranges. The process of radicalization of the anionic and neutral forms of nalidixic acid is accompanied by a TICT-effect.

EXPERIMENTAL

We used the commercial nalidixic acid from Sanoti–Aldrich. The initial nlqH solution with the concentration of 1×10^{-3} M was prepared by dissolving a weighed substance in 100 ml of distilled water. Working concentrations 2×10^{-4} M, 2×10^{-5} M were achieved by sequential dilutions with H_2O or HCl solution (reagent grade). To prepare the samples deionized water was used. All samples were prepared just before the experiment.

In experiments on the laser flash photolysis a modified LFP installation (Luzchem, Canada) was used with excitation by the third harmonic of a Nd:YAG laser Solar TII LF-117 (λ = 355 nm, pulse width 5 ns, the average energy per pulse 1–2 mJ), and probing by an Orion Simplicity light source. All experiments were performed in standard cells (optical path length 1 cm) at 298 K in oxygen-free solutions. The samples for the laser flash photolysis were used till 10% reduction of the absorption maximum.

The calculations of the ground, radical and excited states of nalidixic acid in different protolytic forms were performed using the software GAMESS [8]. Full geometry optimization for each complex was carried out by the Hartree–Fock method in the 6-31G** basis. The analysis of numerical data included the study of frontier orbitals, the electron density distribution in the individual molecular fragments and its changes at the photo-conversion that describe quantitatively the charge transfer in the π-ring system. The results obtained were compared with the values of bond orders and free valence indices, which correlate with the reactivity of the complexes.

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