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PHOTONICS

On the Effect of Excited-State Protolytic Reactions on the Validity of Thermodynamic Relations for Luminescence Spectra

E. E. Volkova[a, b] **and S. A. Tovstun**[a, c, *]

[a] Institute of Problems of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Moscow oblast, 142432 Russia
[b] Faculty of Fundamental Physicochemical Engineering, Moscow State University, Moscow, 119991 Russia
[c] Moscow Institute of Physics and Technology (National Research University), Moscow oblast, Dolgoprudnyi, 141701 Russia
*e-mail: tovstun@icp.ac.ru

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Abstract—In an aqueous solution, fluorescein can exist in the form of several protolytic forms. Transitions between these forms in an excited state greatly complicate the process of studying the luminescent properties of individual components. For such a system, the emission spectrum depends on the excitation wavelength. In this paper, it is shown that for a sample having these properties, the universal Kennard thermodynamic relation for luminescence intensity is valid under any conditions, whereas the less general Kennard–Stepanov relation can be violated.

Keywords: Kennard relation, Kennard–Stepanov relation, fluorescein, protolytic reactions

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INTRODUCTION

In 1926, E.H. Kennard published a paper [1], in which he gave a relation valid for any luminophore in a state of thermodynamic equilibrium. This ratio can be written in the following form [2]:

$$\frac{F(\lambda_{\rm ex},\lambda_{\rm em})}{F(\lambda_{\rm em},\lambda_{\rm ex})}=\frac{F_{\rm B}(\lambda_{\rm em})}{F_{\rm B}(\lambda_{\rm ex})}\frac{(1+n(\lambda_{\rm em}))}{(1+n(\lambda_{\rm ex}))}, \qquad (1)$$

where $F(\lambda_{\rm ex}, \lambda_{\rm em})$ is the luminescence intensity observed at a wavelength of $\lambda_{\rm em}$ upon excitation at a wavelength $\lambda_{\rm ex}$, $F_{\rm B}(\lambda)$ is the blackbody radiation spectrum at a wavelength λ, and $n(\lambda)$ is the average number of particles at an energy level according to Bose–Einstein statistics. In the limit of $kT \gg hc/\lambda$ (c is the speed of light, k is the Boltzmann constant, T is the luminophore temperature, λ is the wavelength) valid at least at room temperature for visible light, Eq. (1) can be simplified as:

$$\ln\left(\frac{{\rm PL}(\lambda,\lambda_0)}{{\rm PLE}(\lambda,\lambda_0)}\frac{\lambda^4}{{\lambda_0}^4}\right)=-\frac{hc}{kT}\left(\frac{1}{\lambda}-\frac{1}{\lambda_0}\right), \qquad (2)$$

where ${\rm PL}(\lambda, \lambda_0)$ is the luminescence spectrum (all spectra in this work are normalized to the number of photons per unit wavelength) upon excitation at a wavelength of λ_0, ${\rm PLE}(\lambda, \lambda_0)$ is the luminescence excitation spectrum detected at a wavelength λ_0. Relationship (2) was theoretically substantiated and generalized to the time domain in [2]. The equation has already been experimentally tested on solutions of diarylethylenes [3], which can simultaneously be in several forms in the ground state, and colloidal quantum dots [2]. In this work, an experimental test is proposed using an aqueous fluorescein solution.

This choice of this luminophore as the object of study is explained as follows. The fluorescein molecule contains three oxygen atoms capable of protonation/deprotonation. By attaching and detaching a proton, the molecule can thus change its luminescent properties. In an aqueous solution in the pH range from 0 to 9, this compound is always present in several forms [4]. These are one cationic, three neutral, two anionic, and one dianionic forms (Fig. 1).

In this study, all the neutral forms (hereinafter simply referred to as the neutral form) are considered as one form, since they always occur in the same proportion in a solution regardless of pH and effectively act as one component in the absorption spectrum. In the anionic form, the phenolate anion can be ignored (the right-hand anion species in Fig. 1), since it is almost undetectable in the aqueous solution. Depending on its pH, the solution may contain a mixture of several forms in a certain ratio. At pH values equal to the negative decimal logarithms of dissociation constants (p$K_{\rm a}$) for certain forms, two forms are present simultaneously in almost identical amounts in the solution. The p$K_{\rm a}$ values for each of the forms were obtained in [5] to be 2.22 for the cation, 4.24 for the set of neutral forms, and 6.28 for the anion.

In addition to the ability to be protonated/deprotonated in the ground state, depending on the pH, the

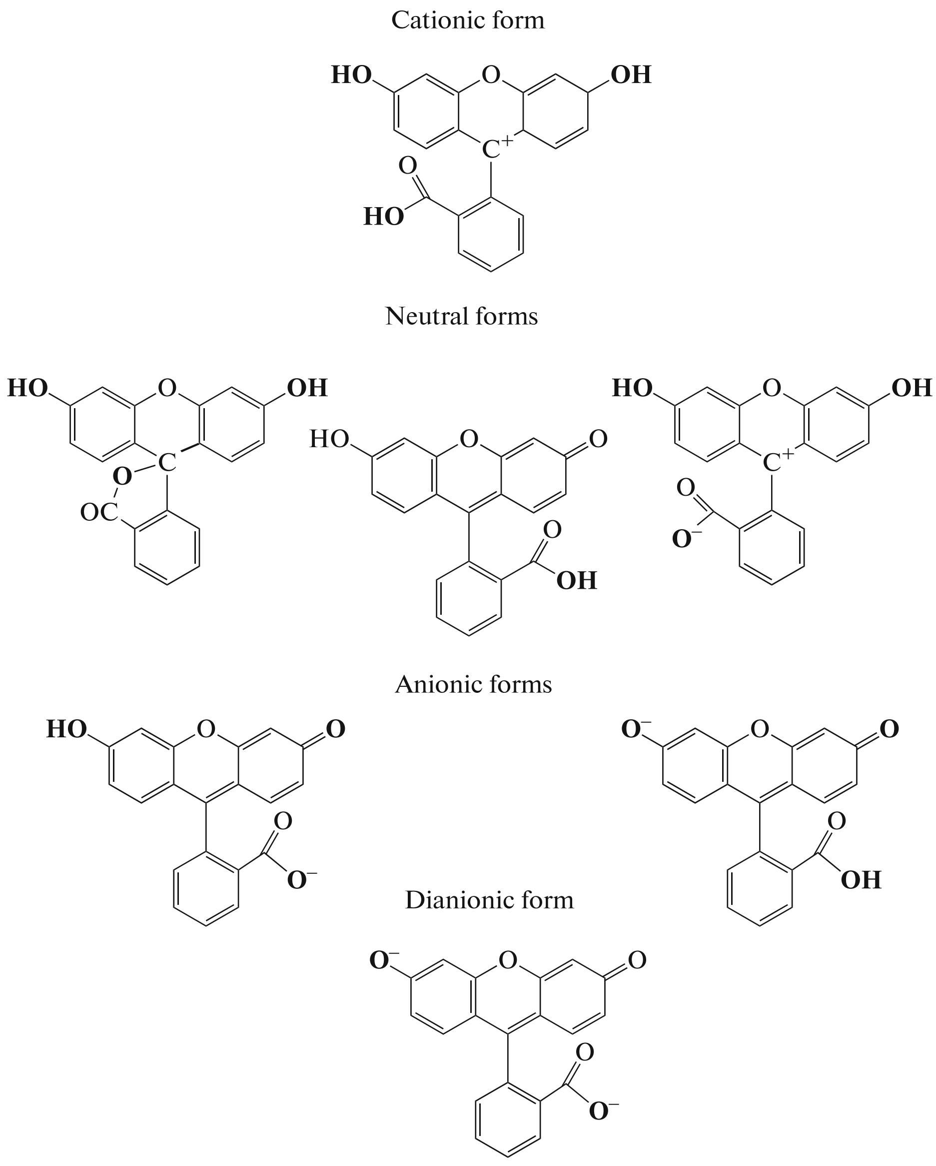

Fig. 1. Forms of fluorescein in an aqueous solution; given in bold italics are the functional groups capable of protonation/deprotonation.

fluorescein molecule is able to transform into another form in an excited state [6]. In fact, the protolytic reaction time for the cation and the neutral form of such a molecule is much shorter than the lifetime of the excited state. Therefore, it is barely possible to observe the emission spectrum of the pure cation and the pure neutral form: in the excited state, they instantly transform into the anion (although the equilibrium at very low pH is shifted toward the cationic form even in the excited state).

Thus, in view of the occurrence of excited-state protolytic reactions, the fluorescein solution is a suitable object for experimental verification of the Kennard relation—it must hold no matter how complex the luminescence mechanism is.

To show that the result obtained is not trivial, we also verified the Kennard–Stepanov relation [7], which, unlike Kennard's law (1), relates emission spectra PL(λ) to absorption spectra ABS(λ):

$$\ln\left(\frac{\mathrm{PL}(\lambda)}{\mathrm{ABS}(\lambda)}\lambda^4\right) = -\frac{hc}{kT}\frac{1}{\lambda} + \mathrm{const}. \tag{3}$$

Relation (3) holds for luminophores in which all luminescence centers are the same; i.e., there is no inhomogeneous broadening, and thermal equilibrium in energy levels in the excited state is established quickly compared with the luminescence lifetime. It can also hold for various luminescence centers if, in the excited state, they can quickly transform into each other or exchange energy. In the case of fluorescein in water, coexistence of its various forms is possible, which can transform into each other in both ground and excited states. It is not entirely clear in advance whether the Kennard–Stepanov relation will be valid in this case, and therefore it makes sense to verify experimentally this assumption.

EXPERIMENTAL

Equipment

Absorption spectra were measured on a Shimadzu UV 3101PC spectrophotometer. Luminescence measurements were carried out on a Shimadzu RF-6000 spectrofluorimeter. In this case, light filters were placed on the paths of the excitation and detection beams, which did not transmit light of higher diffraction orders on the monochromators of the instrument. The measurements were carried out at room temperature in 1-cm fused silica cells. The passband of the excitation and detection monochromators was 3 nm. The weight of the samples was measured on an Ohaus AP-250D analytical balance.

Preparation of Solutions

Three types of buffer solutions were prepared for the study: a phosphate buffer with a concentration of 5.6 mmol/L (by buffer concentration is meant the sum of the concentrations of an acid and a base that compose the buffer) in the pH range from 5.9 to 8, a phosphate buffer in a concentration of 1.11 mol/L with the pH ranging from 4.1 to 5.9, and a citrate buffer of a 0.05 mol/L concentration with the pH ranging from 1.5 to 4.0. To prepare these solutions, sodium hydrogen phosphate and dihydrogen phosphate, sodium citrate, and a 36% hydrochloric acid solution were taken.

For measurements, a stock fluorescein solution with an absorbance of ~15 was prepared. The dye was dissolved in 0.01 M NaOH, after which it was used to prepare solutions with high and low absorbance.

RESULTS AND DISCUSSION

Figure 2 shows the results of measurements of the emission and absorption spectra at several solvent pH values at which one or another form prevails. The obtained absorption spectra demonstrate the following behavior of the sample. At pH 8, the dianionic form prevails in the solution, which is characterized by the absorption spectrum shown by curve *1* in Fig. 2. As the pH decreases to 5.2, the absorbance noticeably decreases and a weakly pronounced second peak appears near 450 nm (Fig. 2, curve *2*)—the anion form prevails in that case. At pH 3.5, the neutral form is present in the largest amount in the solution. Its absorbance is the lowest in comparison with the other forms (Fig. 2, curve *3*). At pH values of about 1.5, the absorption spectrum of the almost pure cation can be observed (Fig. 2, curve *4*). Its absorbance is higher than that of the anionic and neutral forms.

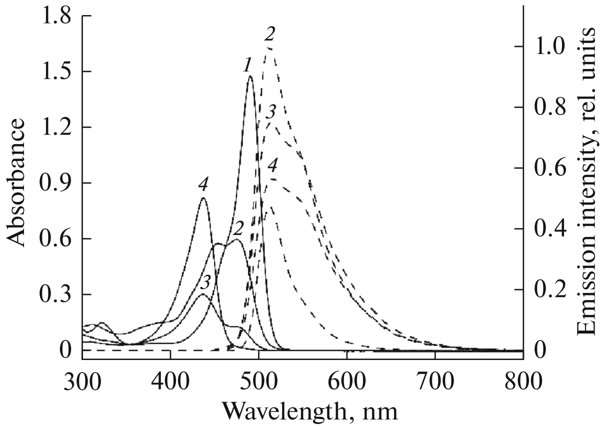

Fig. 2. Luminescence spectra upon excitation at 490 nm (dashed lines) and absorption spectra (solid lines) of aqueous fluorescein solutions for various pH values corresponding to the predominance of a particular protolytic form: (*1*) pH 8 for dianion, (*2*) pH 5.6 for anion, (*3*) pH 3.5 for neutral forms, and (*4*) pH 1.5 for cation. The luminescence spectra are scaled vertically for clarity.

The absorption spectra show the behavior of ground-state fluorescein, whereas the emission spectra can show its behavior in the excited state. The figure shows that the luminescence intensity for different acidity values varies (at the same values of absorbance for each sample). It is known that protolytic reactions occur in excited-state fluorescein [6].

In order to verify the adequacy of the scheme proposed in the literature for protolytic reactions of fluorescein, the excitation/luminescence matrices were analyzed using the PARAFAC method. Figure 3 shows the results of this analysis. As can be seen from Fig. 3, deconvolution of the luminescence excitation spectrum gives three components and that of the luminescence spectrum results in only two. This deconvolution is explained as follows. Due to the weak π–π conjugation between the xanthene moiety of the dye and the benzene ring, the absorption and luminescence spectra of different forms of fluorescein are determined mainly by the degree of protonation of the xanthene moiety. This dye moiety contains two oxygen atoms, which can attach a proton. Due to the symmetry of position of these groups, there are three possible states of protonation of the xanthene moiety of the dye and, hence, three different components in the luminescence excitation spectrum (solid curves in Fig. 3). Hypothetically, there should also be three components for the luminescence spectrum, but one of them, corresponding to the fully protonated xanthene moiety, is almost not manifested in actuality at positive pH values because of rapid proton detachment in the excited state. Therefore, only two components remain in the observed luminescence spectrum

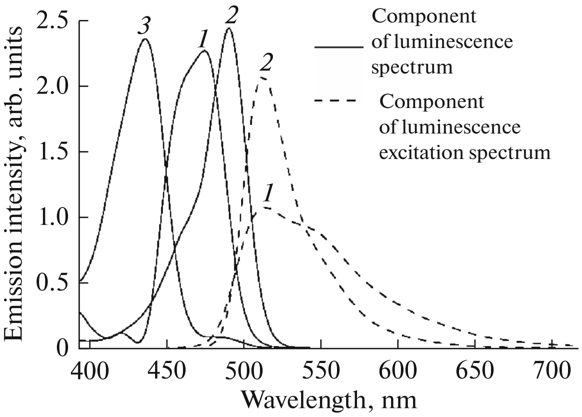

Fig. 3. The elementary luminescence and luminescence excitation spectra obtained as a result of PARAFAC deconvolution of the excitation/luminescence matrices. The figure shows the components for the (*1*) anionic, (*2*) dianionic, and (*3*) cationic forms.

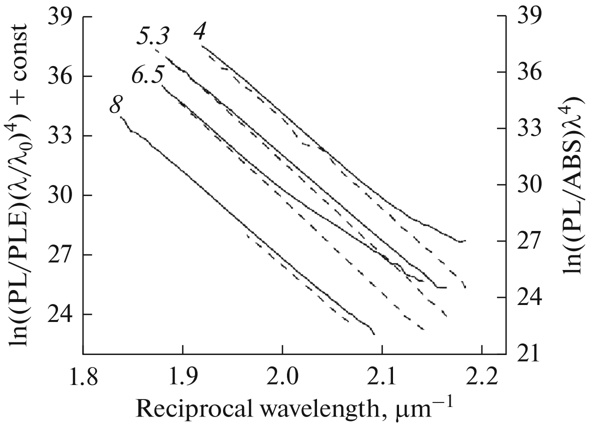

Fig. 4. Comparison of linear transforms of the Kennard (dashed line) and Kennard–Stepanov (solid line) relation for a fluorescein solution at different solvent pH values (indicated above the curves). The transforms of the Kennard relation are shifted along the ordinate axis for clarity, since only their slope is important for the study.

(dashed curves in Fig. 3). Thus, the analysis of the excitation/luminescence matrices has confirmed the mechanism supposed in the literature for excited-state protolytic reactions.

The result of verifying the Kennard and Kennard–Stepanov relations (Eqs. (2) and (3), respectively) is shown in Fig. 4. It is seen that for pH values of 6.5 and 4, the plots according to the Kennard–Stepanov relation do not satisfy the linear law, whereas the data plotted according to the Kennard relation yield straight lines without exception. Approximate values for the slope of these lines were also calculated, which was found to be $-hc/kT$. From these values, the temperature of the solution was calculated. It was found that the deviations from actual values (298 K) did not exceed 5% for the Kennard ratio, whereas those for the Kennard–Stepanov ratio reached 18%.

CONCLUSIONS

The luminescent properties of a fluorescein solution at various pH values of a buffer solvent have been studied, and it has been shown that the excited-state protolytic reactions described in the literature do occur for such a sample. It has also been shown that for a fluorescein solution in the pH range from 2 to 9, the universal Kennard thermodynamic law is always true, but the Kennard–Stepanov thermodynamic relation can be violated.

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