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Spectral properties of BADAN in solutions with different polarities

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ABSTRACT

Spectral characteristics (absorption spectra, excitation and emission spectra of solvatochromic dye 6-bromo-acetyl-2-dimethylamino-naphtalene (BADAN) were studied in solvents with different polarities. Comparison of BADAN fluorescence excitation spectra corrected for the primary inner filter effect and absorption spectra of this dye allowed to conclude that the long-wavelength absorption band of BADAN is determined by the superposition of two BADAN tautomeric forms having different conformation (planar and non-planar) and different distribution of electronic density. The analysis of BADAN excitation and emission spectra has shown that in non-polar solvents (hexane) fluoresces only one dye form which absorbs in more short-wave part of the spectrum than other conformer. In polar solvents (acetonitrile, mixture of acetonitrile/water) both forms fluoresce. It is concluded that the fluorescence of the dye in hexane and short-wavelength component of BADAN fluorescence at excitation in short-wavelength region of absorption spectrum are due to the locally excited state of the planar BADAN molecule. Fluorescence of BADAN in acetonitrile at excitation in long-wavelength part of long-wavelength absorption band and long-wavelength component of BADAN fluorescence spectrum in acetonitrile at excitation in short-wavelength region of absorption spectrum are due to non-planar BADAN conformers with internal charge transfer state.

Introduction

Fluorescent solvatochromic dye BADAN (6-bromo-acetyl-2-dimethylamino-naphtalene) (Fig. 1) is used for studying of the phase transitions in biological membranes, protein conformational changes induced by different factors, proteins interactions with their partners and ligands [1–15]. Furthermore, fluorescence of this dye is used as a recorded signal in biosensor systems [16–21]. The widespread using of BADAN for studying of biological objects is result of high sensitivity of BADAN to the properties of its microenvironment.

The electron density distribution of the ground state of BADAN molecule has a sufficient non-uniform character. According to [22,23] at excitation of BADAN, the dye molecule becomes even more polar: the threefold increasing of its dipole moment was observed. Consequently both ground and excited states of BADAN interact with solvent that is shown in the red shift of BADAN absorption and fluorescence spectra with increasing of solvent polarity [22]. Strong interaction of BADAN molecule in the excited state with solvent molecules is confirmed by the change of shape and position of fluorescence time-resolved spectra in the range of pico to nanoseconds, also [8].

The dependence of BADAN fluorescent characteristics on its environment properties is often explained by use the data about its well-studied structural analogues PRODAN and LAURDAN [4,8,9]. The fluorescence of these dyes has dual character and is described in terms of locally excited state and charge-transfer state in polar solvents [24–28]. There is no single vision of charge-transfer state conformation among researchers: in some works it was suggested that planar molecule fluoresces, while in other it was suggested that non-planar [27,29–38]. Some authors allow the simultaneous existence of PICT (planar internal charge transfer state) and TICT (twisted internal charge transfer state) [8].

The non-planar conformation of BADAN may be the result of:

- dye structural dynamics;
- solvent relaxation;
- hydrogen bonding (carbonyl group of BADAN has an excess charge [9]).

In work [8] it was suggested that some BADAN molecules has non-planar conformation already in ground state.

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It should be noted that the insufficient attention was given to the study of the inherent photophysical properties of the dye. Usually, the analysis of the fluorescence characteristics of BADAN is performed in biological systems (or systems, modeling biological systems) and the obtained data are used for explaining both photophysics of the dye and biological meaning of the studied system at the same time. This complicates the understanding of the nature of the BADAN fluorescence. The purpose of this study was explaining the fluorescence characteristics of the dye in a polar and non-polar environment.

Materials and methods

The fluorescent dye BADAN (AnaSpec, USA), acetonitrile, hexane (Sigma, USA) were used without further purification. The absorption spectra were recorded using a U-3900H spectrophotometer (Hitachi, Japan) in cells 5 × 5 mm from Hellma (Germany). The fluorescence experiments were carried out using Cary Eclipse (Agilent, Australia) spectrofluorimeter. The measurements were made at 23 °C with cells 10 × 10 mm (Starna, USA).

Total fluorescence intensity of BADAN

$$F(\lambda_{ex}) = \int_{\lambda_{em}} F(\lambda_{ex}, \lambda_{em}) d\lambda_{em} \quad (1)$$

was corrected to the primary inner filter effect [39]

$$F_0(\lambda_{ex}) = F(\lambda_{ex})/W \quad (2)$$

where W is correction factor.

Because the fluorescence measurements were performed using the Cary Eclipse spectrofluorimeter, the value of correction factor W was calculated based on ratio

$$W = \frac{\left(1 - 10^{-A_\Sigma}\right)}{A_\Sigma} \quad (3)$$

where A_Σ is the total absorbance of exciting light in the solution $A_\Sigma = \sum_i A_i + A_{abs}$. A_i is absorbance of i-fluorescent component, A_{abs} is absorbance of components which only absorb, but does not fluoresce. In case the solution is monodisperse, i.e. molecules of one substance which are responsible for both absorption and fluorescence present in solution $A_\Sigma = A_{FL}$. In the work [39] it was shown that corrected in such manner value of the total fluorescence intensity is proportional to the product of the absorbance A_{FL} to the quantum yield of fluorescence q when in solution presents one fluorescent substance and corrected total fluorescence intensity equal to sum of the products of these quantities in solution in the presence of several fluorescent substances

$$F_0(\lambda_{ex}) = \sum_i A_{FL,i} q_i \quad (4)$$

The fluorescence emission spectra of BADAN were recorded at λ_{ex} in the range from 250 to 430 nm with step of 5 nm. The fluorescence excitation spectra of BADAN were recorded at λ_{em} in the range from 380 to 500 nm in hexane and from 400 to 560 nm in polar solvents with step of 10 nm. Decomposition of fluorescence spectra of BADAN in acetonitrile was carried out as [40].

Results and discussions

In this work the spectral characteristics of BADAN in non-polar (hexane) and polar (acetonitrile and mixture of acetonitrile/water) solvents were examined. The position and shape of BADAN fluorescence spectrum in hexane (ε = 1.89) are independent of excitation wavelength (Fig. 2A). The fluorescence of the dye in this solvent has one component character and is described by "classical"

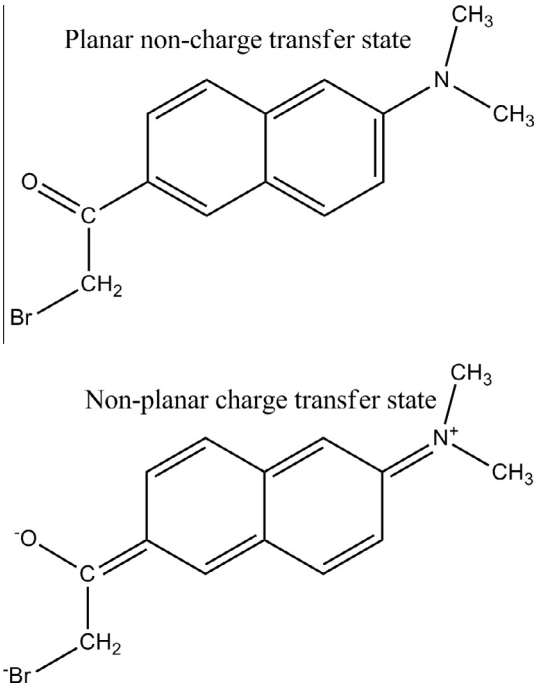

Fig. 1. Chemical structure of fluorescent dye BADAN (6-bromo-acetyl-2-dimethylamino-naphtalene) in ground and charge transfer state.

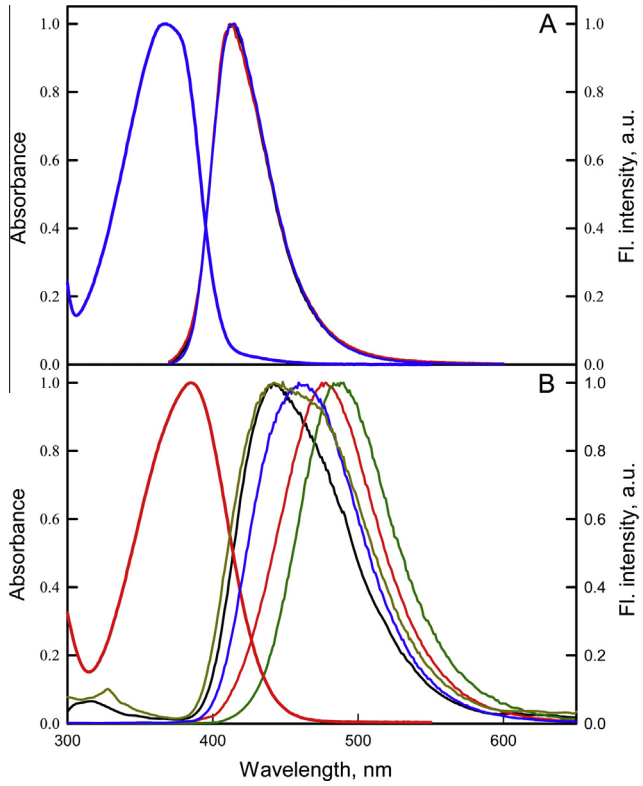

Fig. 2. Fluorescence spectra of BADAN in hexane (Panel A) at excitation wavelength 250 nm (black curve), 300 nm (red curve) and 360 nm (blue curve). Fluorescence spectra of BADAN in acetonitrile (Panel B) at excitation wavelength 250 nm (black curve), 300 nm (dark yellow curve), 340 nm (blue curve), 390 nm (red curve) and 420 nm (green curve). The absorption spectra of BADAN in acetonitrile and hexane are represents red and blue solid curves at panels, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)