4-Dimethylaminochalcone as fluorescent probe: effect of the medium polarity on relaxation processes in the excited state

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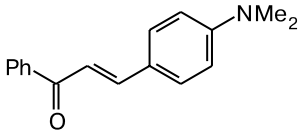
Relaxation processes in a 4-dimethylaminochalcone molecule after excitation with a light pulse of duration 70 fs were studied. During 0.4—1 ps after excitation, an absorbance of an excited state S_1 with a maximum at ~460 nm is formed in both polar and nonpolar media. Subsequent relaxation processes depend on the polarity of the medium. In nonpolar hexane, the 4-dimethylaminochalcone molecule transits to the triplet state having an absorption maximum at 570 nm (lifetime longer than 600 ps) for 20 ps. In polar aprotic acetonitrile, the absorbance at 460 nm decreases slowly (during hundreds of picoseconds), indicating that the molecules return to the ground state. The induced emission from the level S_1 in a region of 520—550 nm and fluorescence from the same level with a maximum at 537 nm are also observed in acetonitrile. Thus, a reason for a sharp decrease in the fluorescence yield on going from polar to nonpolar media was found. The mechanism of fluorescence quenching of 4-dimethylaminochalcone in nonpolar media is confirmed by the data on phosphorescence. The phosphorescence of 4-dimethylaminochalcone is observed at −196 °C in nonpolar solvents, indicating a triplet excited state, while no phosphorescence is revealed in polar solvents.

Key words: fluorescent probe, 4-dimethylaminochalcone, femtosecond dynamics, polarity of medium, triplet state.

4-Dimethylaminochalcone (DMAC) as a fluorescent probe is used for studying biological membranes and lipoproteins.[1—6] It is poorly soluble in water and, hence, binds to lipids in the presence of membranes or lipoproteins.[1]



DMAC

In polar solvents, the quantum yield of fluorescence can reach 0.26; however, the fluorescence of DMAC in water is almost completely quenched due to the formation of a hydrogen bond (other protic solvents quench DMAC flluorescence in the same manner).[3,4] When binding to proteins or lipids, a DMAC molecule is shielded from water, and the fluorescence yield can increase to 0.1 and higher. However, if the molecule has "drown" in the lipid depth and got into a nonpolar environment, the yield decreases to 0.01 and lower. Thus, it seems clearly why polar protic solvents quench fluorescence, while reasons for fluorescence quenching in nonpolar media are unknown. In this work, we attempted to elucidate this problem.

Experimental

4-Dimethylaminochalcone was synthesized and kindly presented by B. M. Krasovitskii (Institute for Single Crystals, National Academy of Sciences, Kharkov, Ukraine).[7] Acetonitrile, 95% EtOH, methylcyclohexane, and hexane (Aldrich) were used.

The femtosecond dynamics of transient absorption spectra of DMAC was studied on an experimental system based on a femtosecond CPM dye laser designed at the N. N. Semenov Institute of Chemical Physics of the Russian Academy of Sciences.[8] The "pump—probe" spectroscopy technique was used. The second harmonic from the main sequence of pulses generated by the system was used as pump pulses. The bearing wavelength of a pump pulse was 308 nm, and the duration was 70 fs. Probe pulses were carried out in the wavelength interval from 400 to 590 nm using supercontinuum pulses generated in a cell filled with distilled water. To avoid the influence of molecular rotation, the light from the pump and probe pulses was linearly polarized and their polarization axes were oriented at the magic angle (55°) to each other. The interval of time delays was 600 ps. The "pump—probe" transient absorption method and our procedure for processing of recorded spectra of photoinduced absorption, which takes into account spectral features of probe pulses, have been described previously.[9]

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 8, pp. 1607—1610, August, 2004.

1066-5285/04/5308-1670

Stationary absorption spectra were measured with a Beckman DU-7 spectrophotometer (USA) in quartz cells with the optical path length from 0.2 to 5 cm at ~20 °C.

Phosphorescence spectra were measured on a Hitachi MPF-4 spectrofluorimeter (Japan) with a phosphorescence attachment at −196 °C. Both solvents (nonpolar methylcyclohexane and polar ethanol) produce a clear solid on freezing down to −196 °C.

Results and Discussion

The quantum yield of DMAC fluorescence in hexane, which was used as a nonpolar solvent, does not exceed 0.01, while MeCN was used as a polar aprotic solvent (fluorescence yield 0.11).

Dynamics of the excited state of a DMAC molecule in the nonpolar medium. The absorption spectrum of DMAC in hexane contains a long-wave band with a maximum at 383 nm (Fig. 1); pulse excitation was carried out at the edge of this band at 308 nm. This π—π*-transition is caused by the intramolecular charge transfer from the dimethylamino group acting as a donor to the electron-acceptor fragment, carbonyl group.[10—13]

Control experiments demonstrated that DMAC molecules do not form dimers at the concentrations used (50 μmol L^{-1} and lower): in a range of DMAC concentrations in hexane from 230 to 0.6 μmol L^{-1}, the stationary absorbance of the solution remains proportional to the DMAC concentration, and the shape of the absorption spectrum remains unchanged.

After a DMAC molecule was photoexcited by pump pulses of duration 70 fs, transient absorption spectra are recorded (Fig. 2, *a*). They are likely caused by the light absorption from the Franck—Condon excited sublevels of DMAC. During the time about 1 ps, an absorption band with a maximum at 465 nm is formed, which corresponds, most likely, to transitions from a level S_1 to higher levels S_n.

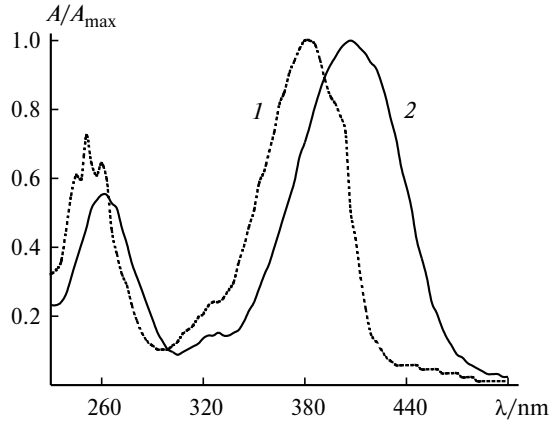


Fig. 1. Normalized absorption spectra of DMAC in hexane (*1*) and MeCN (*2*) at 20 °C.

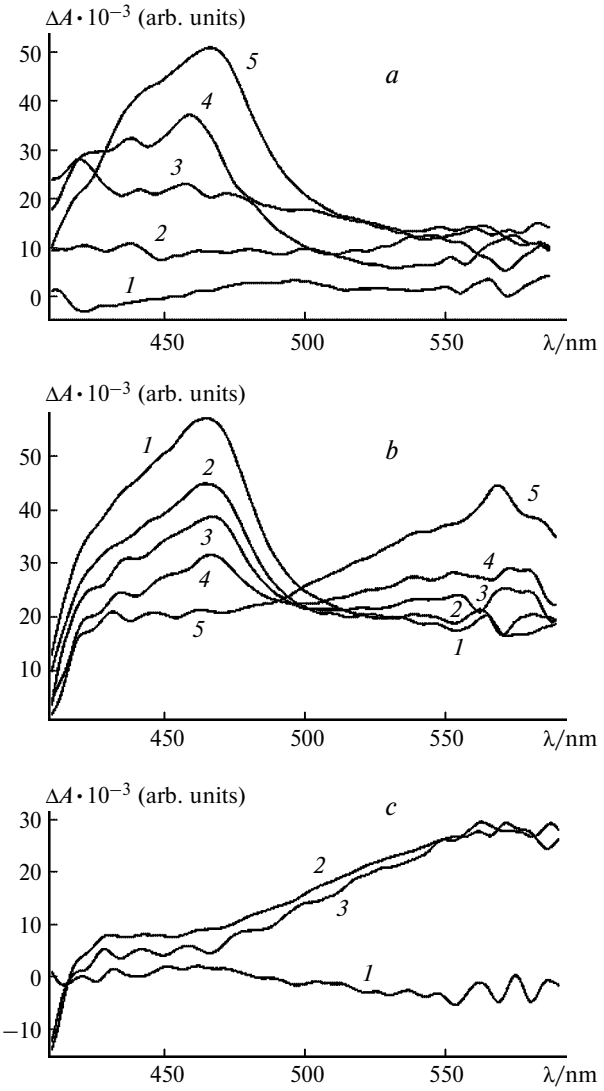


Fig. 2. Differential absorption spectra of DMAC in hexane (50 μmol L^{-1}) upon pulse excitation with λ = 308 nm. Delay time of a probe pulse: −0.3 (*1*), 0 (*2*), 0.1 (*3*), 0.2 (*4*), and 0.5 ps (*5*) (*a*); 1 (*1*), 2 (*2*), 3 (*3*), 5 (*4*), and 15 ps (*5*) (*b*); −0.1 (*1*), 200 (*2*) and 600 ps (*3*) (*c*).

After 1 ps, the intensity of the absorption band at 465 nm begins to decrease. A new absorption band appears simultaneously at 570 nm (Fig. 2, *b*). This suggests that the DMAC molecules that transited within 1 ps to the state with the absorption maximum at 465 nm transit during the next 20 ps to a state with an absorption maximum at 570 nm.

In hexane, DMAC molecules fluoresce with a maximum at 436 nm and a very low yield <0.01.[14] The calculated natural lifetime of the excited DMAC molecule in the S_1 state is ~5000 ps.[15] Therefore, the real lifetime in the S_1 state in hexane cannot exceed 5000 ps × 0.01 = 50 ps. This indirectly confirms the assumption that the

band at 465 nm corresponds to the absorption of the molecules in the S_1 state.

The lifetime of the absorption band at 465 nm does not exceed 20 ps, whereas the absorption intensity at 570 nm does not decrease during the whole observation period for 600 ps (Fig. 2, *c*). It is most likely that DMAC molecules transit from the S_1 state to a long-lived triplet state; the band at 570 nm corresponds, therefore, to the triplet-triplet absorption. The same is indicated by a negative band in the differential absorption spectrum at 400 nm, which is distinctly detected 600 ps after excitation (see Fig. 2, *c*) and caused, evidently, by a decrease in the population of the state S_0, which does not repopulate for 600 fs.

In the case when the S_1 state is deactivated by both crossing to the triplet state and radiationless internal conversion to the state S_0, a recovery of the population of this state should be expected. However, the presence of the intense negative band at 400 nm (see Fig. 2, *c*) shows that the transition from the S_1 state to the triplet is the main route of deactivation of the excited state S_1 in the DMAC molecule in a nonpolar medium.

The data obtained make it possible to estimate the order of rate constants of deactivation of the excited state S_1 in a nonpolar medium: the rate constant of the radiative transition $S_1 \rightarrow S_0$ is equal to $2 \cdot 10^8$ s^{-1}, the rate constant of the transition $S_1 \rightarrow T$ is $\sim 3 \cdot 10^{11}$ s^{-1}, and the rate constant of the nonradiative conversion $S_1 \rightarrow S_0$ is $\ll 3 \cdot 10^{11}$ s^{-1}.

Dynamics of the excited state of a DMAC molecule in a polar medium. The results of experiments on femtosecond absorption spectroscopy of a DMAC solution in the polar aprotic solvent MeCN are shown in Fig. 3. As can be seen from the data in Fig. 3, *a*, after excitation an absorption band at 400 nm is formed for 0.2—0.3 ps. During the next 0.5—0.6 ps, this bands shifts to the long-wave region by 60 nm, which corresponds to the absorption of the S_1 state in nonpolar hexane.

The lifetime of the band at 460 nm is ~500 ps (see Fig. 3, insert). The fluorescence of DMAC with a quantum yield of 0.11 is observed simultaneously. The decay time of this fluorescence can be estimated as τ = 5000 ps × 0.11 = 550 ps. Thus, in polar MeCN the lifetime of the S_1 state is 500—550 ps, while in nonpolar hexane it is shorter than 20 ps. The disappearance of the absorbance at 460 nm is not accompanied by the appearance of any new band, unlike its behavior in hexane. No triplet state of DMAC is formed in the polar solvent.

It should also be noted that in MeCN the negative absorption band at 400 nm is not virtually observed 500 ps after a laser pulse: probably, the population of the S_0 state has recovered.

The differential spectra recorded 0.5 ps after excitation (see Fig. 3, *a*, *b*) exhibit a negative band at

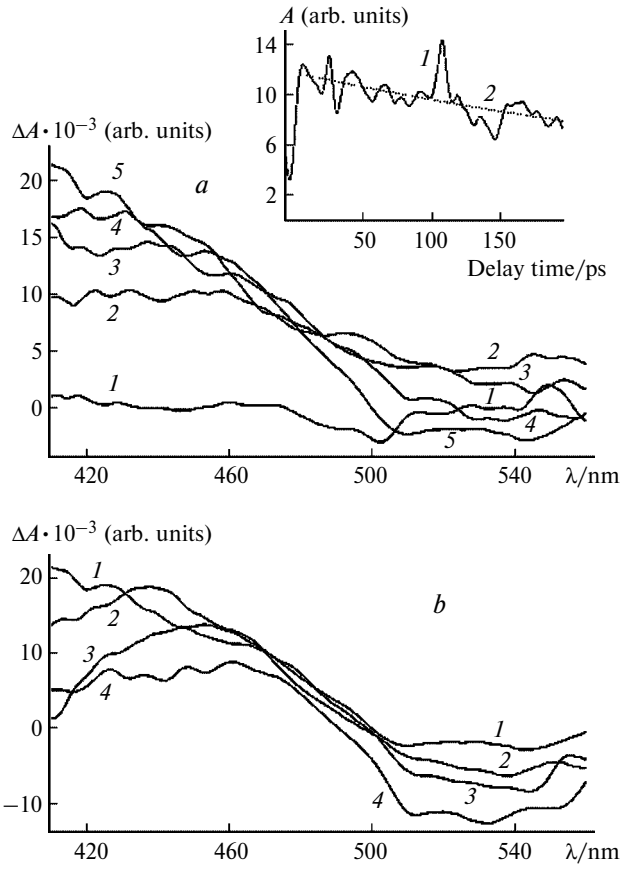


Fig. 3. Differential absorption spectra of DMAC in MeCN (25 μmol L^{-1}) upon pulse excitation with λ = 308 nm. Delay time of a probe pulse: −0.4 (*1*), 0 (*2*), 0.1 (*3*), 0.2 (*4*), and 0.3 ps (*5*) (*a*); 0.3 (*1*), 0.4 (*2*), 0.5 (*3*), and 0.6 ps (*4*) (*b*). Insert: kinetics of the DMAC differential absorbance (λ = 460 nm) in the picosecond scale upon the pulse excitation of DMAC in MeCN (25 μmol L^{-1}) with λ = 308 nm at 20 °C (*1*). Line *2* is a result of approximation by an exponential dependence.

520—550 nm, which was absent from the absorption spectrum of DMAC in hexane (see Fig. 1). The lifetime of this negative band corresponds to the lifetime of the S_1 state, *i.e.*, ~500 ps, and the position of its maximum corresponds to the fluorescence (537 nm in MeCN). It is most likely that this is the induced emission by DMAC molecules in the S_1 state due to the probing light.

The fluorescence yield in polar aprotic media is much higher than that in nonpolar media; however, it does not exceed 0.26 at room temperature, and no transition to the triplet state is observed. This indicates both radiative and nonradiative transitions $S_1 \rightarrow S_0$.

The data obtained make it possible to estimate the order of rate constants of deactivation of the excited state S_1 in polar MeCN: the rate constant of the radiative transition $S_1 \rightarrow S_0$ is equal to $2 \cdot 10^8$ s^{-1}, the rate constant of the transition $S_1 \rightarrow T$ is $\ll 2 \cdot 10^9$ s^{-1}, and the rate constant of the nonradiative conversion $S_1 \rightarrow S_0$ is $\sim 2 \cdot 10^9$ s^{-1}.

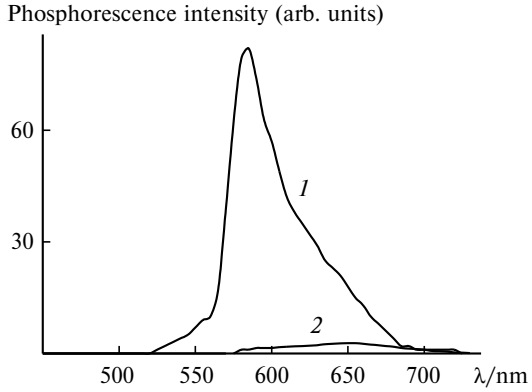


Fig. 4. Phosphorescence spectra of DMAC (0.15 μmol L^{-1}) in methylcyclohexane (*1*) and EtOH (*2*) at —196 °C.

Phosphorescence of DMAC in nonpolar and polar media. The results of femtosecond spectroscopy are confirmed by experiments on phosphorimetry of DMAC solutions in methylcyclohexane and EtOH (Fig. 4). In methylcyclohexane at —196 °C, DMAC phosphoresces. The positions of maxima in the absorption spectra of DMAC, the phosphorescence excitation spectra, and the fluorescence spectra almost coincide, *i.e.*, the emission appears due to light absorption by a DMAC molecule. The phosphorescence maximum lies at ~580 nm for all nonpolar solvents under study: hexane, cyclohexane, methylcyclohexane, and *o*-xylene. In polar solvents (EtOH, MeCN, acetone), no significant phosphorescence was recorded.

Thus, the presence of DMAC phosphorescence in nonpolar media and its absence in polar solvents agree with the result of studying the dynamics of photoinduced absorption: in nonpolar media, DMAC molecules transit from the S_1 state to the triplet state, while in polar media triplets are not formed.

The authors thank S. Ya. Umanskii and A. I. Kokorin for discussion and valuable remarks.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 02-03-32428).

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Received November 12, 2003;
in revised form February 27, 2004