

9-Diphenylaminoacridines as Molecular Fluorescent Chemosensors for Determining Polar Solvent and Amine Vapors

V. A. Sazhnikov, V. M. Aristarkhov, S. K. Sazonov, A. I. Vedernikov, S. P. Gromov, and M. V. Alfimov

Photochemistry Center, Russian Academy of Sciences, ul. Novatorov 7a, Moscow, 119421 Russia

e-mail: sazhnikov@yandex.ru

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Abstract—The results of study of the photophysical properties of three fluorophores from the 9-diphenylaminoacridine series in solutions are presented. It has been shown that the photoinduced synthesis of 9-diphenylaminoacridines in polymer films and on the surface of polymeric micro- and nanoparticles can be used for detection of polar solvent and amine vapors.

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Studying the properties of acridine and its derivatives, in particular 9-aminoacridine and 9-anilinoacridine, is of interest largely because of their numerous applications as bioactive molecules in biology and medicine owing to the ability of the acridine cycle to form π -complexes with nucleic acid bases [1–3]. It was found that the formation of ternary 9-anilinoacridine/DNA/enzyme complexes via further complexation of the aniline moiety with proteins is also possible [4].

Much attention has been given to the spectral-and-luminescent properties of 9-arylaminoacridine derivatives and the acridinium ion characterized by intramolecular electron transfer from the donor moiety (e.g., dimethylaniline) to acridine, which acts as the acceptor moiety, upon electronic excitation [5, 6]. The dependence of the fluorescence spectra of such donor–acceptor compounds on the polarity of the medium was used to design sensors for determining small amounts of water in organic solvents [7]. It should also be noted that the feasibility of the formation of intramolecular electron-transfer states with a very long lifetime was proved for the 9-mesityl-10-methylacridinium ion [8–10].

Previously [11], it was found that UV irradiation of diphenylamine and tetrabromomethane solutions in toluene or hexane yields 9-diphenylaminoacridine along with triphenylmethane dyes. It was also shown that the use of tetrabromomethane and various diphenylamine derivatives allows for the photoinduced synthesis of acridine, phenylacridine, 9-ditolylaminoacridine (9-DTAA), etc. in solutions [12]. A possible reaction mechanism of the photoinduced synthesis of these compounds is described in [11–14].

The compounds synthesized, in particular 9-DTAA, exhibit well-defined solvatochromic properties [15].

In this paper, we show that 9-diphenylaminoacridines, introduced into a polymer film or produced in a film by photoinduced synthesis, are molecular chemosensors useful as fluorescent indicators of electron- or proton-acceptor compounds, in particular chlorinated solvents, ammonia, and pyridine, in the vapor phase.

EXPERIMENTAL

All the solvents (Aldrich) were of the grade “for UV spectroscopy” and were used without further purification. The reactants used for the photoinduced synthesis of 9-diphenylaminoacridine fluorophores in solutions and polymer layers were diphenylamine and its derivatives that bear the methyl (ditolylamine) or the dimethylbenzyl (4,4'-bis(alpha,alpha-dimethylbenzyl)diphenylamine) group in the *para*-position. Tetrabromomethane (TBM), diphenylamine (DPA), di-*p*-tolylamine (DTA), and (4,4'-bis(alpha,alpha-dimethylbenzyl)diphenylamine) (DMBDPA) (Fluka, Aldrich, Merck) was used without further purification. The choice of DTA and DBMDFA as reactants was based on the fact that the synthesis of nonfluorescent triphenylmethane dyes is blocked in this case [11–13]. According to the reaction mechanism described [11–13], HBr is released during the photoinduced synthesis and results in protonation of the photoreaction products.

The synthesis procedure described in [11–13, 15] comprises irradiating a solution of an appropriate amine and TBM in hexane with full light from a DRSh-1000 lamp for 4–6 h, dissolving the precipitate in benzene, washing with an aqueous sodium bicarbonate solution, evaporating in a vacuum, and purifying the product by recrystallization or column chromatography. The structural formulas of synthesized compounds **1–3** are shown in Fig. 1. The melting

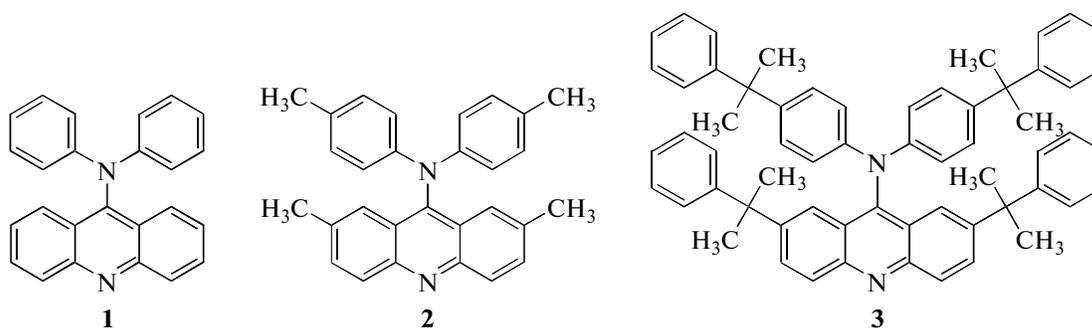


Fig. 1. Structural formulas of synthesized 9-diphenylaminoacridines.

points of crystalline *N,N*-diphenylacridine-9-amine (9-diphenylaminoacridine, 9-DPAA, **1**), 2,7-dimethyl-*N,N*-dimethyl-*N,N*-di-*p*-tolylacridine-9-amine (9-ditolylaminoacridine, 9-DTAA, **2**), and 2,7-bis(2-phenylpropan-2-yl)-*N,N*-bis(4-(2-phenylpropan-2-yl)phenyl)acridine-9-amine (DBAA, **3**) are 225, 261, and 179°C, respectively.

The absorption spectra of solutions of **1–3** were recorded on a Shimadzu UV-3101 PC spectrophotometer, and the fluorescence spectra were measured with a Shimadzu RF-5301 PC spectrofluorometer. The standards used for measuring the fluorescence quantum yields were fluorescein and Rhodamine 123; their quantum yields were assumed to be 0.9. The fluorescence lifetime was measured by the phase modulation technique with a Fluorolog-3 instrument (Jobin Yvon, France).

As a polymer matrix for preparing sensor layers based on neutral fluorophores **1–3**, unplasticized polystyrene ($M = 280000$, Aldrich) was used. To prepare samples, solutions of **1–3** (10^{-3} mol/L) in tolu-

ene were mixed with a 10% polymer solution in toluene and the mixture was applied onto a glass substrate with a dispenser and dried. The average thickness of the films was 20–40 μm . Thin polystyrene films were prepared by the spin coating technique using an SCS 6720D device.

The substrates with the samples were placed in a saturated solvent vapor atmosphere, and a change in the fluorescence signal intensity was monitored using an SD-2000 fiber-optic spectrofluorimeter and Ocean Optics fluorescent probes. A light-emitting diode with $\lambda = 375$ nm was used as a light source.

To run the photoinduced synthesis of compounds **1–3** in polystyrene layers, solutions of the reactant amines and tetrabromomethane in toluene (10^{-1} mol/L) were applied onto a glass substrate with a dispenser or by spin casting and irradiated with UV light.

RESULTS AND DISCUSSION

The absorption spectrum of **2** in hexane and its fluorescence spectra in a number of solvents are given in [15]. The absorption spectra of **1** and **3** in hexane are almost identical to the spectrum of **2**, which has absorption maxima at 290, 360, and 450 nm. All the three compounds are characterized by a small bathochromic shift (of 300 to 500 cm^{-1}) of the long-wavelength band on passing to a polar solvent (THF, acetone, DMSO). The molar absorption coefficients at maximum of the long-wavelength band in all solvents are about 11000 $\text{L mol}^{-1} \text{cm}^{-1}$ on average for **1** and **2** and about 15000 $\text{L mol}^{-1} \text{cm}^{-1}$ for **3**.

The absorption spectra of the protonated forms of **1–3** were prepared by adding hydrochloric acid to dilute solutions in methanol (Fig. 2). It can be seen that the maxima of the long-wavelength bands for the protonated forms **1⁺–3⁺** lie in the region of 520–540 nm. Thus, the protonation of the nitrogen atom in the acridine cycle leads to quite a strong bathochromic shift (about 80 nm) of the long-wavelength absorption bands of **1–3**. The molar absorption coefficient is increased for **1** and **2** (to 19000 and 16000 $\text{L mol}^{-1} \text{cm}^{-1}$, respec-

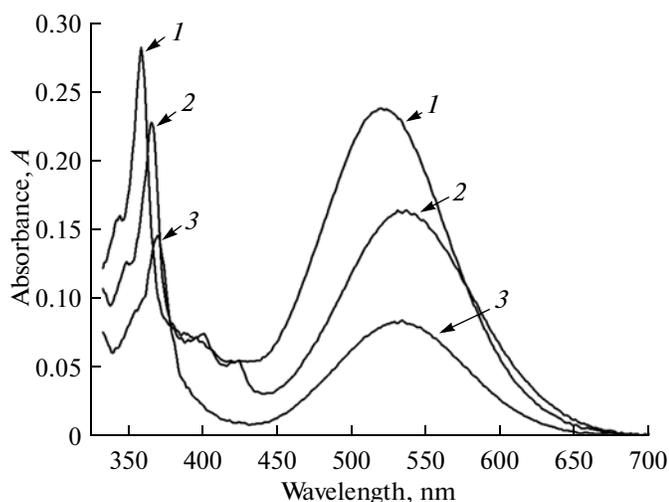


Fig. 2. Absorption spectra of protonated 9-diphenylaminoacridines **1⁺–3⁺** in acidified methanol ($c = 10^{-5}$ mol/L, $c(\text{HCl}) = 0.5$ mol/L, the arrows show band assignment).

tively) and decreased for **3** (to $7000 \text{ L mol}^{-1} \text{ cm}^{-1}$) by protonation.

Unlike the case of absorption spectra, the fluorescence maxima of neutral 9-diphenylaminoacridines **1–3** are bathochromically shifted to a significant extent (by 3000 to 5000 cm^{-1}) on passing from cyclohexane ($\lambda_{\text{max}} = 470 \text{ nm}$) to polar solvents. As an example, Fig. 3 shows the fluorescence spectra of **2** in the chlorinated solvents tetrachloromethane (TCM), trichloromethane (TrCM), and dichloromethane (DCM). For comparison, the spectrum in toluene is also shown. As seen from Fig. 3, the fluorescence band of 9-DTAA in nonpolar TCM is shifted toward shorter wavelengths ($\lambda_{\text{max}} = 515 \text{ nm}$) relative to weakly polar toluene ($\lambda_{\text{max}} = 537 \text{ nm}$), whereas there are significant bathochromic shifts (65 – 70 nm) for TrCM and DCM having a large dipole moment. In [15] it was shown that the strong solvatochromic effect for **2** can be explained in terms of an increase in the dipole moment from 2 to 12 debyes upon the transition to the first singlet excited state. It is obvious that a similar change in the dipole moments by excitation should be assumed for compounds **1** and **3**.

The table presents data on the quantum yields and the fluorescence lifetimes of **2** in different solvents. Similar data were obtained for **1** and **3**. It is seen that an increase in solvent polarity, which is characterized by the Reichardt index $E_T(30)$ [16], leads to a decrease in both the quantum yield and the lifetime of fluorescence of **2**. Calculation by the conventional relationships $k_r = \phi_F/\tau_F$ and $k_{nr} = (1 - \phi_F)/\tau_F$ shows that the observed changes in ϕ_F and τ_F with the increasing of solvent polarity correlate with the decrease in the radiative transition rate constant k_r and the increase in the nonradiative transition rate constant k_{nr} , which is the sum of the internal conversion and intersystem crossing rate constants. By analogy with the case of solvatochromic Nile Red [17], this behavior can be qualitatively explained in terms of two basic factors resulting from the bathochromic shift of the maximum

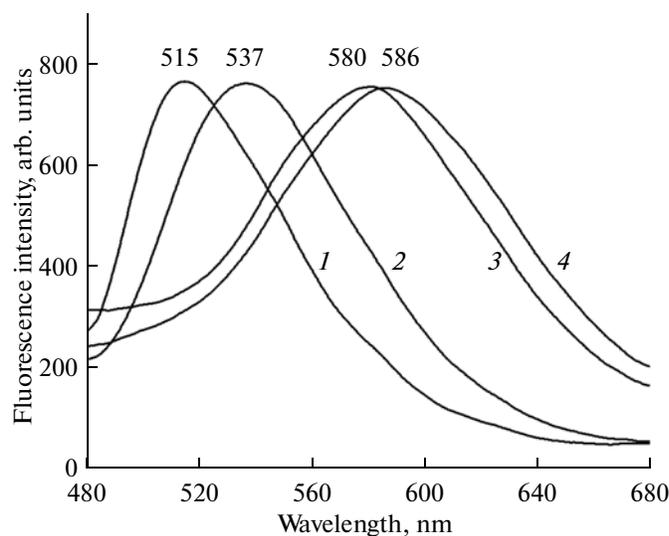


Fig. 3. Normalized fluorescence spectra of a 9-DTAA (**2**) solution in (1) tetrachloromethane, (2) toluene, (3) trichloromethane, and (4) dichloromethane. The figures refer to the position of the band maxima.

of the fluorescence band, the decrease in the probability of radiative transitions with the increasing wavelength (Strickler–Berg equation [18]) and the increase in the probability of the nonradiative decay of the first excited state by the enhancement of vibronic interactions with the ground state on reducing the energy gap between the states [19]. The contribution of intersystem crossing processes to the decay of the first singlet state of 9-diphenylaminoacridines **1–3** calls for a separate investigation.

Note that no appreciable fluorescence in the red region of the spectrum could be detected in the case of excitation to the long-wavelength absorption band of protonated molecules $\mathbf{1}^+$ – $\mathbf{3}^+$ in acidified methanol solutions. Most likely, this is a consequence of both relatively low quantum yields of fluorescence and its

Quantum yields of fluorescence, fluorescence lifetimes, and rate constants of radiative and nonradiative transitions for compound **2**

Solvent	$E_T(30)$, kcal/mol	ϕ_F	τ_F , ns	k_r	k_{nr}
				10^7 s^{-1}	
Cyclohexane	30.9	0.57	29.3	1.95	1.47
Toluene	33.9	0.30	27.0	1.11	2.59
Dioxane	36.0	0.27	24.2	1.12	3.02
THF	36.2	0.15	18.9	0.79	4.50
Chloroform	39.1	0.13	18.0	0.72	4.83
Acetone	42.2	0.06	12.5	0.48	7.52
DMSO	45.1	0.02	10.2	0.20	9.61
Acetonitrile	45.6	0.03	7.8	0.38	12.4
Propanol	48.4	0.008	7.2	0.11	13.78

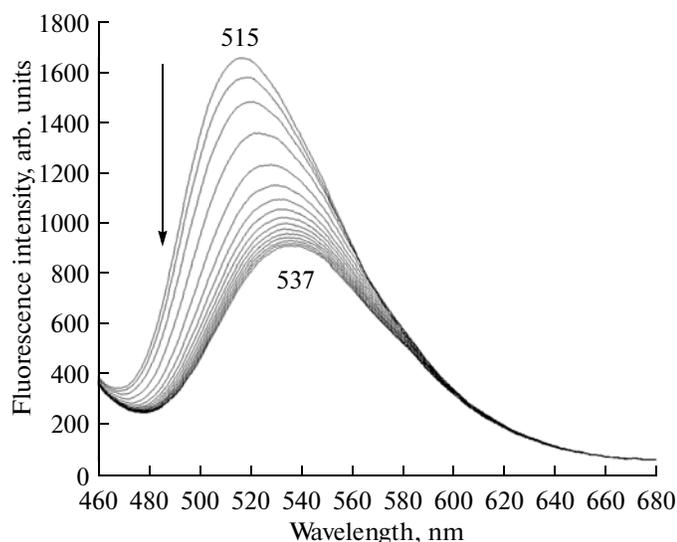


Fig. 4. Change with time of the fluorescence spectrum of a PS film doped with 9-DTAA (**2**) during holding in saturated toluene vapor (curves were recorded at 3-s intervals).

additional quenching due to hydrogen bonding with alcohol molecules.

It was found that polystyrene (PS) films doped with compounds **1–3** exhibit quite bright fluorescence peaked at 510–520 nm when excited with LED at $\lambda = 375$ nm.

The data presented in Fig. 4 show that holding these films in saturated toluene vapor leads to a shift of the fluorescence maximum to 537 nm, which corresponds to the maximum of fluorescence of **2** in pure toluene (Fig. 3). The observed changes most likely reflect the process of completion of the solvation shell about the molecules of **2** to the shell characteristic of pure toluene by toluene molecules diffusing into the film. The fluorescence variation process is fully reversible: after removing the sample from the toluene vapor atmosphere, the spectrum is quickly (20 s) and fully restored.

Figure 5 shows the change in the fluorescence spectrum of the PS film after its holding for several minutes in a saturated trichloromethane vapor atmosphere. It can be seen that as CHCl_3 molecules diffuse into the film, the fluorescence intensity decreases and the maximum of the fluorescence band simultaneously shifts to a significant extent toward longer wavelengths (from 515 to 564 nm), although the shift is smaller than in the chloroform solution (shifting to 580 nm). Note that the shift to 580 nm cannot be attained even upon a very long exposure. The intensity decay kinetics is almost linear in character.

It is obvious that this behavior can be explained in terms of partial solvation of fluorophore **2** by polar TrCM molecules. The observed spectral changes as a whole are reversible too. However, during the evaporation of TrCM molecules from the film, the reverse shift

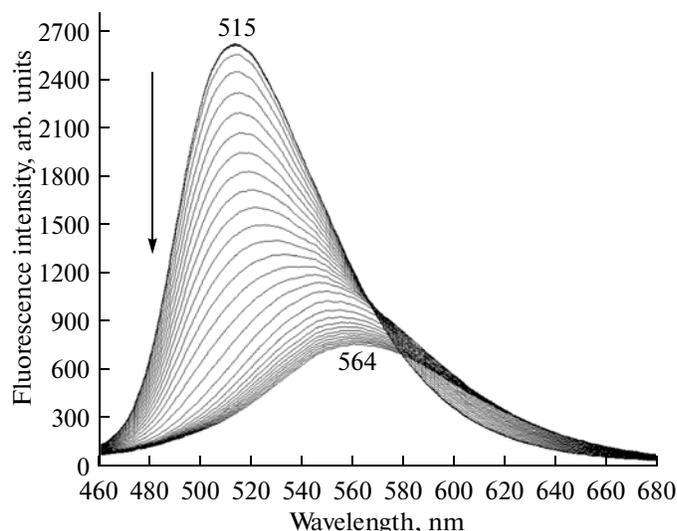


Fig. 5. Change with time of the fluorescence spectrum of a PS film doped with 9-DTAA (**2**) during holding in saturated trichloromethane vapor (curves were recorded at 3-s intervals).

of the band to shorter wavelengths is fast enough (30 s) only for the region from 560 to 530 nm and the final comeback from 530 nm to the initial spectrum with the maximum at 515 nm takes a longer time (to 100–300 s). This is presumably due to the fact that the energy of interaction of the 9-DTAA molecule with one or two TrCM molecules remaining in the solvation shell has a relatively large value.

The spectral change processes and the rate curves for dichloromethane are similar to those for trichloromethane. The maximum of the 9-DTAA fluorescence band is shifted stronger (to 574 nm) by saturation with DCM vapor than in the case of TrCM, but the observed shift is also smaller than that in the DCM solution (586 nm).

Unlike the case of polar TrCM and DCM, holding the PS–DTAA film in vapor of nonpolar tetrachloromethane (TCM) only leads to a uniform decrease in fluorescence intensity without changing the position of the fluorescence band maximum. By itself, this finding is of interest in relation to the sensor properties of PS layers doped with **1–3** because it means that any TCM vapor present in air can be distinguished from a DCM or TrCM vapor by the character of change in the fluorescence spectrum.

As noted above, the photoinduced synthesis of compounds **1–3** from DMF, DTA, and DMBDFA with TBM can be carried out in hexane or toluene solutions at room temperature [11]. It was also found that the photoinduced synthesis reactions occur in polystyrene films as well if they contain the reactant amine and TBM in sufficient concentrations. More recently, this process was studied in more detail by Budyka et al. [14]. Figure 6 shows that the spectral changes during the reactions resulting from the UV

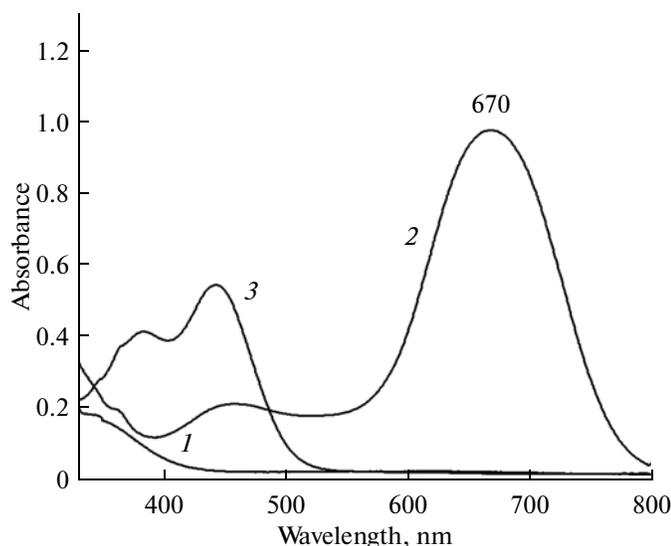


Fig. 6. Absorption spectra of a PS-film containing DPA and tetrabromomethane (1) before and after (2) irradiation with UV light and (3) holding in ammonia vapor.

irradiation of PS films containing DPA and TBM with concentrations of 10^{-2} – 10^{-1} mol/L are similar to those that occur in the solutions [11–13].

The reactants in the PS films form DPA/TBM charge-transfer complexes, which have the absorption spectrum at wavelengths shorter than 400 nm. By UV irradiation, the film is colored as a result of the formation of both primary photoproducts having an absorption maximum at 670 nm and, apparently, protonated 9-DPAA 1^+ with an absorption maximum at about 530 nm. Over time, the initial spectrum (curve 2) transforms into a spectrum characteristic of neutral 9-DPAA (curve 3). This process can be greatly accelerated by warming the samples with hot air or exposing them to ammonia vapor.

It should be noted that the protonated products 9-DPAA $^+$ (1^+) and 9-DTAA $^+$ (2^+) seem to have a relatively low fluorescence quantum yield in the case when the counterion is the anion Br^- . However, it was found that 9-DBAA $^+$ (3^+) bearing bulky substituents in the *para*-position has quite a high quantum yield under these conditions. Figure 7 shows time changes in the fluorescence intensity of the PS film in which the photoinduced synthesis of 3^+ takes place under exposure to light. In the presence of amine or pyridine vapor, this film changes the fluorescence color from red to green.

It should be noted that in the above case of the action of TCM vapor on the PS layers with neutral 9-DTAA, a new, though less intense fluorescence band appears in the red region of the spectrum with a maximum at 630 nm along with the reduction in the intensity of green fluorescence (Fig. 8). The long-term exposure of PS films to TCM vapor (10 min or more)

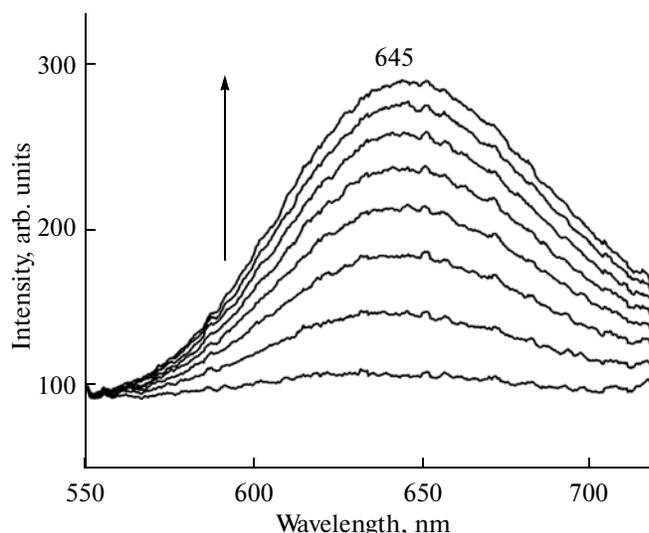


Fig. 7. Change with time of the fluorescence spectrum of 3^+ in the PS film containing the reactants DMBDPA (10^{-1} mol/L) and TBM (10^{-1} mol/L) during irradiation with a light-emitting diode at $\lambda_{\text{max}} = 375$ nm. The curves were recorded at intervals of 10 s.

results in almost complete conversion of green into red fluorescence.

The most likely explanation is the assumption that the red luminescence is due to the appearance of the protonated form 2^+ , which readily gives up the proton to ammonia or an organic base having a higher proton affinity compared with **2**. Protonated 2^+ can be formed via the addition to **2** of the HCl molecule that is generated by the reaction of photoinduced electron transfer from **2** to CCl_4 and the subsequent irreversible dissociation of the radical anion CCl_4^- to the Cl^- ion and the CCl_3 radical.

Thus, when Cl^- is a counterion for 2^+ , fluorescence in the red region of the spectrum is easy to detect and can be used for analytical purposes. Figure 9 shows the conversion of the initial weak red fluorescence of the film into bright green fluorescence by the action of saturated pyridine vapor.

These data indicate that the synthesized compounds **1–3** can form charge-transfer complexes with TCM, like the reactant arylamines. This in turn means that the reactions of formation of photoproducts **1–3** from arylamines and tetrabromomethane are self-sensitized, since the photoreaction products themselves are sensitizers of the subsequent formation of molecules **1–3**.

It was shown that the effect of self-sensitization leads to an exponential increase in the amount of the photoproducts formed at the site of irradiation of the film with visible light (e.g., 630-nm light from a helium–neon laser). In principle, this effect can be used to dramatically increase the local fluorophore concentration in the required place of the film.

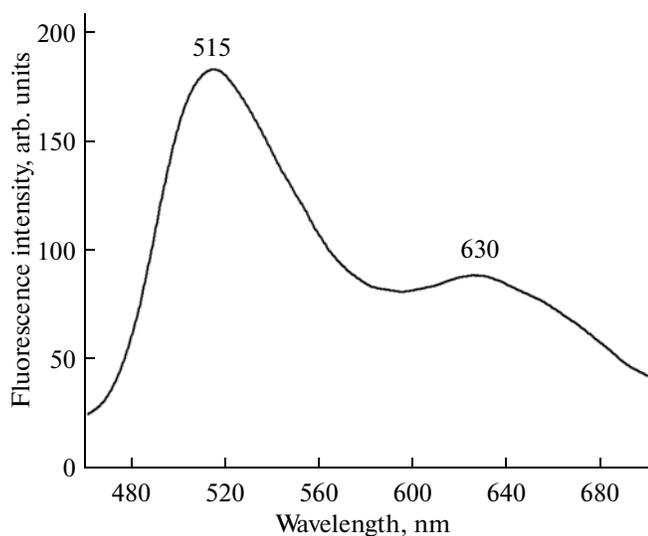


Fig. 8. Fluorescence spectrum of the PS-film with 9-DTAA (**2**) after irradiation with a light-emitting diode at $\lambda_{\text{max}} = 375$ nm for a few minutes in tetrachloromethane vapor.

It is noteworthy that the photoinduced synthesis of compounds **1–3** can be accomplished not only in thin polymer layers, but also on the surface of polymeric micro- and nanoparticles, which can be further used to fabricate matrices for sensor elements by means of a jet printing device [20].

CONCLUSIONS

It has been shown that the 9-diphenylaminoacridine compounds 9-DPAA, 9-DTAA, and 9-DBAA (**1–3**) immobilized in polymer films can be used as a fluorescent molecular sensors whose fluorescence intensity and color vary in the presence of polar organic solvents or amines. The reactions of the photoinduced formation of fluorophores **1–3** can be used for designing specialty sensor materials, since the formation of an indicator fluorophore in the polymer layer can be initiated immediately before the measurement to obtain the indicator fluorophore at the desired site in the desired concentration. It is also possible to fabricate sensor layers suitable for visual indication by change of fluorescence color in the presence of an analyte.

Since 9-diarylaminoacridines change their fluorescence properties during the protonation–deprotonation reaction, they can be used as acid–base indicators in various media.

Synthesized diphenylaminoacridines **1–3** can be considered 9-anilinoacridine derivatives and, therefore, can be of use in biomedical research. Probably, the two phenyl rings can enhance their ability to form complexes with proteins in comparison with 9-anilinoacridines. The presence of donor and acceptor moi-

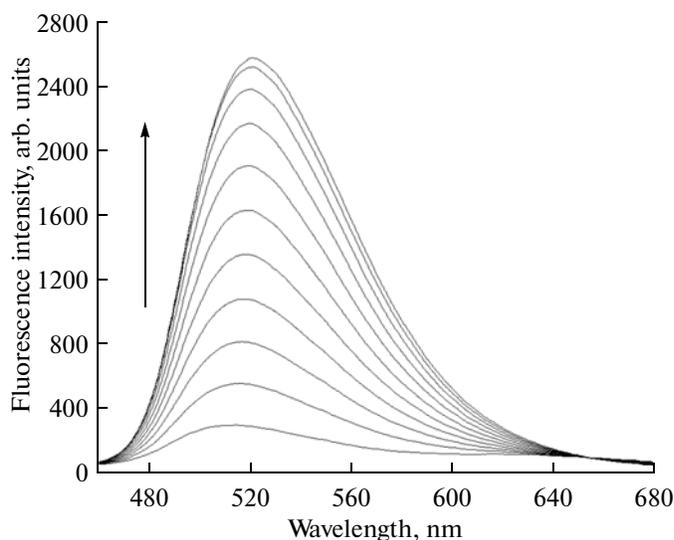


Fig. 9. Change in the fluorescence spectrum of the PS film with 9-DTAA⁺ (**2**⁺) in pyridine vapor (spectra recorded at intervals of 5 s).

eties in molecules of **1–3** and the diversity of their fluorescent properties are their additional advantage.

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