Journal of Fluorescence, Vol. 15, No. 1, January 2005 (©2005)
DOI: 10.1007/s10895-005-210-1

Dipole Moments of 4′-Aminoflavonol Fluorescent Probes in Different Solvents

N. A. Nemkovich,[1,2,4] V. G. Pivovarenko,[3] W. Baumann,[1] A. N. Rubinov,[2] and A. N. Sobchuk[2]

Received March 17, 2004; accepted August 14, 2004

Electrooptical absorption measurements (EOAM) were used to measure the dipole moments of the normal form of 4′-(dimethylamino)-3-hydroxyflavone (FME), and 4′*N*-(15-azacrown-5)-3-hydroxyflavone (FCR) in 1,4-dioxane, toluene, and cyclohexane. With these probes excited-state intramolecular proton transfer (ESIPT) takes place. For comparison, the dipole moments of 4′-(dimethylamino)-3-metoxyflavone (FME3ME), for which ESIPT is lacking, were measured, too. For all three probes the ground (μ_g) and excited Franck-Condon state (μ_e^{FC}) electrical dipole moments are parallel to each other and also parallel to the transition dipole moment. The electrical dipole moments of FCR, FME, and FME3ME in their ground state have values within the range (12.0–17.7) $\times$ 10^{-30} C m. Upon optical excitation, the dipole moments increase by (41.9–52.9) $\times 10^{-30}$ C m. The value of the change of the dipole moment vector $\Delta^a\mu$ with excitation to the Franck-Condon state and the value of the vector μ_e^{FC} for FCR and FME are practically independent on the solvent polarity. From this point of view and due to large values of the dipole moments FCR and FME are very promising probes for the investigation of the distribution of the local polarity in biological systems using site-selective excitation of the different sites. Our steady-state fluorescence studies on FME and FCR have demonstrated a high spectral sensitivity of the normal form to such solvent characteristics as polarity.

KEY WORDS: Flavonols; fluorescent probes; dipole moments; electrooptical absorption measurements.

INTRODUCTION

The biophysical and biomedical application of fluorescence spectroscopy is increasing. For example, the fluorescent probe method now is widely used for studying cells, proteins, and tissues [1–3]. The local dielectric properties of cells, proteins, and tissue are of great importance for diagnostic and investigation of their function. The best known method for the estimation of the local dielectric constant in complex macromolecules is based on using standard equations, describing the dependence of the position of the electronic spectra of fluorescence probes on the dielectric constant, refractive index of the medium, and on the dipole moments of the probe in its ground and excited states. If the dipole moments in the relevant electronic states are known, the local dielectric constant may be determined by these equations from the spectral position of the electronic spectra [4–10].

Electrooptical absorption and emission measurements in solution (molecular Stark-effect spectroscopy in terminology of [11,12]) provide valuable information about the values and directions of the dipole moments and

[1] Institute of Physical Chemistry, University of Mainz, Jakob Welder-Weg 11, 55099 Mainz, Germany.
[2] BI Stepanov Institute of Physics, National Academy of Sciences of Belarus, F. Skaryna Ave. 68, 220072 Minsk, Belarus.
[3] Department of Chemistry, National Taras Shevchenko University, Volodymyrska 64, 01033 Kiev, Ukraine.
[4] To whom correspondence should be addressed. E-mail: nemkovic@uni-mainz.de or nemkov@ifanbel.bas-net.by

ABBREVIATIONS: FME, 4′-(dimethylamino)-3-hydroxyflavone; FME3ME, 4′-(dimethylamino)-3-methoxyflavone; FCR, 4′-N-(15-azacrown-5)-3-hydroxyflavone; 1-AN, 1-phenylnaphthylamine; ESIPT, excited-state intramolecular proton transfer; EOAM, electrooptical absorption measurements.

polarizabilities in the ground and excited states of probe molecules [13–16]. The strong advantage of the electrooptical measurements in comparison with the various existing solvent shift variants is that the dipole moments are determined in a single solvent, and hence it is possible to study the influence of polarity of the environment on the values of the dipole moments. This is very essential for selection of the suitable fluorescent probes for study dielectric interactions in biological systems.

Usually the probe molecules have several binding sites with different polarity and their electronic spectra are inhomogeneously broadened [17]. Hence, the selective excitation of molecules located at different sites becomes possible by changing the frequency of the exciting light. In Ref. [10], from the position of the normalized quantum fluorescence spectrum of 1-phenylnaphthylamine (1-AN) the dielectric constant of human erythrocyte ghosts was calculated under different frequencies of excitation, i.e. at different spatial locations 1-AN inside a membrane. But, if the values of the dipole moments themselves are dependent on the polarity of the medium, the error with the determination of the local dielectric constant by the above-mentioned method may be quite large. Therefore the dipole moments of fluorescent probe molecules must be studied in solvents with different polarity.

In this paper, we present the results from electrooptical absorption measurements (EOAM) on the ground and excited Franck-Condon state dipole moments of the normal form of 4′-(dimethylamino)-3-hydroxyflavone (FME) and 4′-*N*-(15-azacrown-5)-3-hydroxyflavone (FCR) in 1,4-dioxane, toluene, and cyclohexane. Due to excited-state intramolecular proton transfer (ESIPT) the spectra of FME and FCR observed in various solvents have two well resolved fluorescence bands, one of which corresponds to the emission of the normal form (N*), and the other one to the flavonol phototautomer (T*) [18–23]. Our steady-state fluorescence studies on FME and FCR have demonstrated a high spectral sensitivity of the normal form to such solvent characteristics as polarity. For comparison, we also measured the dipole moments of 4′-(dimethylamino)-3-metoxyflavone (FME3ME), for which ESIPT is lacking.

EXPERIMENTAL

Flavonol Synthesis

The flavonols (Fig. 1) have been synthesized from 2-hydroxyacetophenone and the corresponding benzaldehydes by the Algar–Flynn–Oyamada reaction [24] and purified by means of repeated recrystallization or column chromatography. All flavonols were homogeneous at thin-layer chromatography on Silufol UV-254 plates in chloroform-methanol (98:2, 95:5, 9:1 or 85:15, v/v) with following detection by UV excitation at 254 nm and 360 nm wavelengths. Their structures have been confirmed by quantitative elemental analysis, nuclear magnetic resonance (NMR), UV–Visible, and infrared (IR) spectrometry.

All solvents were obtained from Merck and were dried prior to use in the electrooptical measurements.

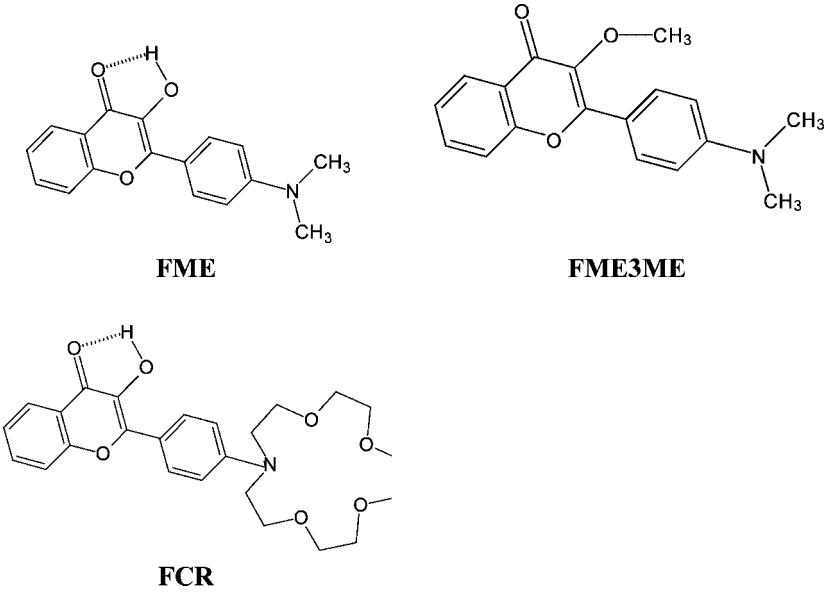

Fig. 1. Fluorescence probes studied in this work.

The steady-state absorption and emission spectra were recorded by a spectrophotometer Perkin Elmer (LS 50B) and SFL-112A spectrofluorimeter (Solar, Minsk), respectively.

Electrooptical Absorption Measurements

To determine the ground- and excited-state dipole moments of the normal form, we used EOAM. Experimental details on the electrooptical methods have been reviewed extensively [13,14]. Using Liptay's formalism [25] the effect of an external electric field E_f on the molar absorption coefficient $\kappa(\tilde{\nu})$ can be described by a quantity L, which is defined by

$$L = L(\tilde{\nu}, \chi) = [\kappa^E(\tilde{\nu}, \chi) - \kappa(\tilde{\nu})] \Big/ \left[\kappa(\tilde{\nu}) E_f^2\right] \quad (1)$$

where κ^E is the molar absorption coefficient in the presence of an applied electric field, κ is the same without applied electric field, χ is the angle between the direction of E_f and the electric field vector of the incident light and $\tilde{\nu}$ is the wavenumber. For a homogeneously broadened absorption band L is given by the following equation

$$L = Dr + [1/6]Es + Frt + Gst + Hru + Isu \quad (2)$$

where the parameters r and s are determined by the angle χ, and the quantities t and u depend on the first and second derivatives of the absorption spectrum

$$r = (2 - \cos^2\chi)/5 \quad (3)$$

$$s = (3\cos^2\chi - 1)/5 \quad (4)$$

$$t = (1/hc)(\kappa/\tilde{\nu})^{-1} \mathrm{d}(\kappa/\tilde{\nu})/\mathrm{d}\tilde{\nu} \quad (5)$$

$$u = (1/2h^2c^2)(\kappa/\tilde{\nu})^{-1} \mathrm{d}^2(\kappa/\tilde{\nu})/\mathrm{d}\tilde{\nu}^2 \quad (6)$$

The coefficients D, E, F, G, H, I are connected with intrinsic properties of the solute molecules. Neglecting some explicit polarizability terms D to I can be written as

$$D = \left(f_e^2/kT\right)\mathbf{R}^{(1)}\mu_g \quad (7)$$

$$E = (f_e/kT)^2\left[3(\mathbf{m}_a\mu_g)^2 - \mu_g^2\right] + \left(f_e^2/kT\right)\left(3\mathbf{R}^{(2)} - 2\mathbf{R}^{(1)}\right)\mu_g \quad (8)$$

$$F = \left(f_e^2/kT\right)(\mu_g\Delta^a\mu) + f_e^2\mathbf{R}^{(1)}\Delta^a\mu \quad (9)$$

$$G = \left(f_e^2/kT\right)(\mathbf{m}_a\mu_g)(\mathbf{m}_a\Delta^a\mu) + \left(f_e^2/2\right)\mathbf{R}^{(2)}\Delta^a\mu \quad (10)$$

$$H = f_e^2(\Delta^a\mu)^2 \quad (11)$$

$$I = f_e^2(\mathbf{m}_a\Delta^a\mu)^2 \quad (12)$$

where k is the Boltzman constant, T is the temperature, $\mathbf{m}_a$ is the unit vector in the direction of the transition moment for absorption, μ_g is the equilibrated ground state dipole moment vector, $\Delta^a\mu$ is the change of the dipole moment vector upon excitation to the considered Franck-Condon excited state. The vectors $\mathbf{R}^{(1)}$ and $\mathbf{R}^{(2)}$ are related to the transition polarizability of the considered transition and describe the effects due to the electric field dependence of the transition moment. The field correction is done by the cavity field factor f_e [13].

The quantity $L(\tilde{\nu}, \chi)$ in the present work was determined for two values of the angle χ($\chi = 0$ and $\chi = \pi/2$) and for a set of wavenumbers within the first absorption band. Then the coefficients (8)–(12) and their standard deviations were obtained from fitting of the experimental L values by the program SYSTAT Version 7.0 according to Eq. (2).

RESULTS AND DISCUSSION

Fluorescence Spectra of Flavonols

Our studies on FME and FCR have demonstrated their high spectral sensitivity to such solvent characteristics as polarity. First of all this sensitivity appears in the steady-state fluorescence spectra as different spectral position, intensity and width of the flavonol normal form (N*) emission band. The fluorescence spectra of FME in various aprotic solvents are shown in Fig. 2. As it is seen, the N* form display an essential positive solvatofluorochromic effect. From measured spectra, we recovered the fluorescence bands of the normal and phototautomer form using the Gaussian approximation

$$I(\nu) = \begin{cases} I_0 \exp\left(-(\nu - \nu^{\max})^2/\sigma_1^2\right), & \nu \le \nu^{\max} \\ I_0 \exp\left(-(\nu - \nu^{\max})^2/\sigma_2^2\right), & \nu > \nu^{\max} \end{cases}$$

where $\nu^{\max}$ and $\sigma_{1,2}$ are peak frequency and dispersions, respectively. The quality of the fluorescence spectrum fitting was monitored by the reduced statistic χ_s^2 and by the visual inspection of residuals. Figure 3 displays the example of recovering of the two bands, which correspond to fluorescence of the normal form and phototautomer, from the experimentally measured emission spectra of FME in acetonitrile.

The dependence of the fluorescence peak frequency of the normal and phototautomer form, obtained in the above mentioned way, on the reaction dielectric field parameter *F(ε, n)* [26] is shown on Fig. 4. As follows from Fig. 4, the frequency shift of the normal fluorescence peak $\nu_{em}^{\max}$ is strongly dependent on the reaction field parameter. For example, $\nu_{em}^{\max}$ changes from 22785 cm^{-1} in paraffin oil to 19513 cm^{-1} in acetonitrile (Table I). The large solvatofluorochromic effect in the fluorescence spectra of flavonols is a real evidence of an essential change of the electric dipole moment of the probe molecules after

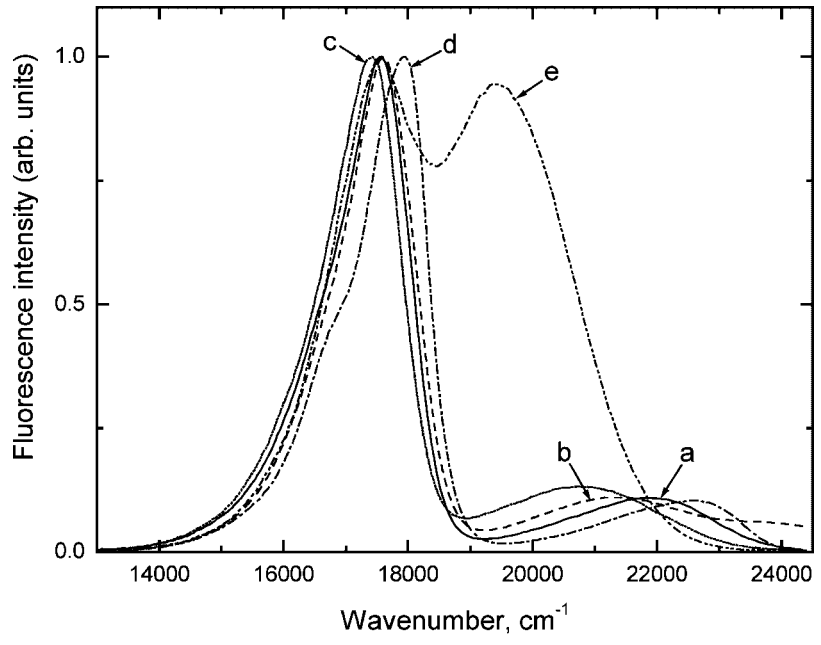

Fig. 2. Normalized fluorescence spectra of FME in different solvents: (a) 1,4-dioxane (— — —), (b) fluorobenzene (— — —), (c) tetrahydrofuran (···), (d) paraffin oil (— . —), (e) acetonitrile (— —). [FME] = 5 μM; $T = 23^{\circ}$C.

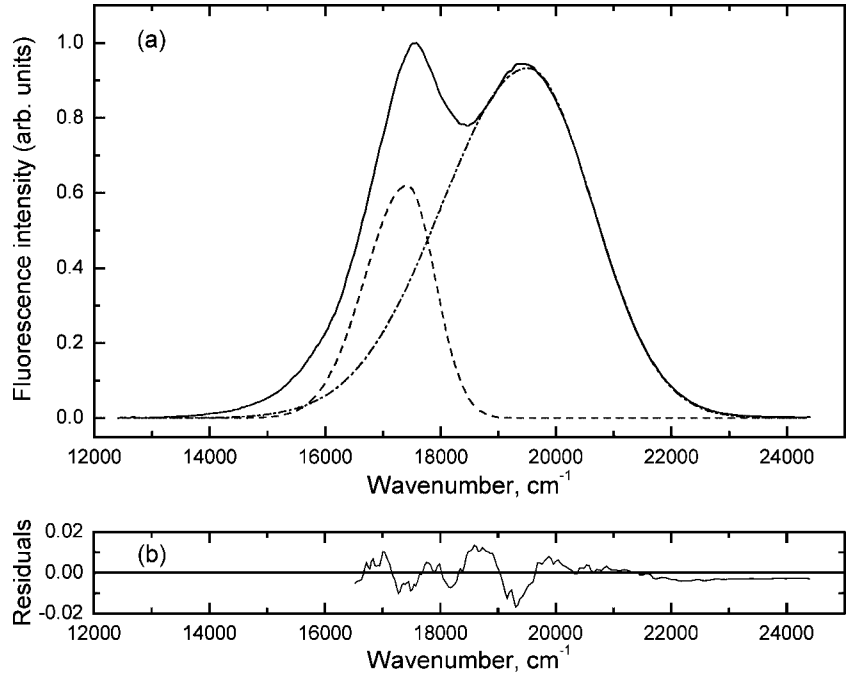

Fig. 3. Fluorescence emission spectra of FME in acetonitrile. (a) Experimentally measured fluorescence spectra (———), recovered fluorescence spectra of the normal (— — —) and phototautomer (— — —) form in the Gaussian approximation $I(\nu) = \begin{cases} I_0 \exp(-(\nu - \nu^{\max})^2/\sigma_1^2), & \nu \le \nu^{\max} \\ I_0 \exp(-(\nu - \nu^{\max})^2/\sigma_2^2), & \nu > \nu^{\max} \end{cases}$, where $\nu^{\max}$ and $\sigma_{1,2}$ are peak frequency and dispersions, respectively. Lower panel (b) shows plot of residuals. $\lambda_{ex} = 394$ nm; [FME] = 5 μM; $T = 23^{\circ}$C.

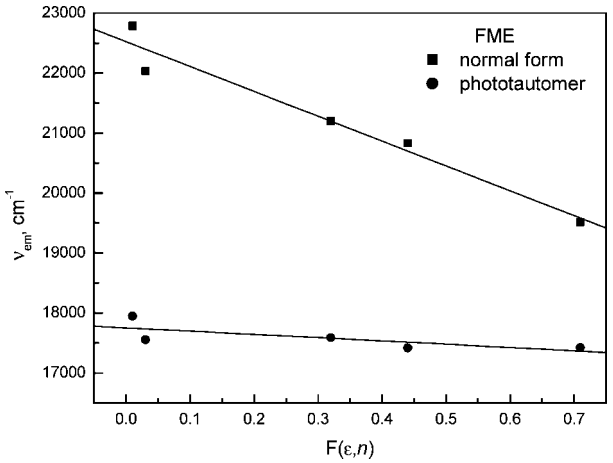

Fig. 4. Dependence of the normal (■) and phototautomer (●) fluorescence peak frequencies for FME as a function of the reaction field parameter [26] $F(e, n) = (\varepsilon + 2) - (n^2 - 1)/(n^2 + 2)\varepsilon$, where ε and n are dielectric constant and refractive index, respectively.

excitation. This also means, that the local dielectric constant of the surrounding medium may be determined from the position of the probe electronic spectra, if the dipole moments of the fluorophore are known.

As follows from our measurements the ratio of the I_N^*/I_T^* fluorescence peak intensities increases with increasing reaction field parameter $F(\varepsilon, n)$ of the solvent (Table I). The latter effect, as is known [18], is connected with the change of the probability of the direct and reverse ESIPT and may be used for two-wavelength ratiometric detection of changes in the local polarity in biological systems.

Dipole Moments of Flavonols

The electrooptical absorption spectra of all three flavonols are accurately reproducible, and have the maximum at the red edge of the absorption spectrum. As an example, the experimental data points of the electrooptical absorption spectra of FCR in 1,4-dioxane are shown in Fig. 5.

Table I. Ratio (I_N^*/I_T^*) of the Fluorescence Peak Intensities and the Frequency of the Fluorescence Peak (ν_{em}^{max}) of Different Forms of FME in Various Solvents. $F(\varepsilon, n)$—the Reaction Field Parameter [26]

Solvent	$F(\varepsilon, n)$	I_N^*/I_T^*	$\nu_{em}^{max}(cm^{-1})$, normal form	$\nu_{em}^{max}(cm^{-1})$, phototautomer
Paraffin oil	0.01	0.100	22785	17948
Dioxane	0.03	0.107	22033	17556
Fluorobenzene	0.32	0.108	21199	17591
Tetrahydrofuran	0.44	0.133	20829	17419
Acetonitrile	0.71	1.503	19513	17423

Note. The frequency of the fluorescence peak (ν_{em}^{max}) of normal form and phototautomer was recalculated by the special program of decomposition the experimentally measured spectra on two bands with nonsymmetrical Gaussian shape.

From our measurements, follow that for all probes in all solvents the coefficient F equals G and coefficient H equals I, within the experimental error. As an example, in Table II the results of EOAM on FME in toluene solutions at $T = 298$ K are presented. This means that $\mathbf{m}_a \| \mu_g \| \Delta^a\mu$. The same result follows from the slope of the function $L(\nu, \chi = 0) = f[L(\nu, \chi = \pi/2)]$ which for solute molecules with C_n-symmetry is given in good approximation by the simple linear relationship [27,28]:

$$L(\nu, \chi = 0) = AL(\nu, \chi = \pi/2) + Bf_e^2\mu_g^2/6k^2T^2 \quad (13)$$

where $A = (1 + 2\cos^2\theta)/(2 - \cos^2\theta)$; $B = (3\cos^2\theta - 1)/(2 - \cos^2\theta)$ and θ is the angle between $\mathbf{m}_a$ and μ_g.

Figure 6 shows the respective plot of $L(\nu, \chi = 0)$ versus $L(\nu, \chi = \pi/2)$ for FCR in 1,4-dioxane. The points represent the experimental data and the line is their approximation by a linear regression. From the linearity of the function $L(\nu, \chi = 0) = f[L(\nu\chi = \pi/2)]$ for FCR, FME, and FME3ME follows that the first absorption band of the studied probes is sufficiently homogeneous and that the angle between the vectors $\mathbf{m}_a$ and μ_g is constant over the measured wavenumber interval in according with Eq. 13. From the slope of the function $L(\nu, \chi = 0) = f[L(\nu\chi = \pi/2)]$ for FCR, FME, and FME3ME we found that the coefficient $A \cong 3$. From Eq. (13) follows that in this case $\mathbf{m}_a \| \mu_g \| \Delta^a\mu$.

Using the symmetry condition $\mathbf{m}_a \| \mu_g \| \Delta^a\mu$ the values of the dipole moments μ_g and $\Delta^a\mu$ were calculated from

$$\mu_g = (kT/f_e)\sqrt{\frac{E - 6D}{2}} \quad (14)$$

$$\Delta^a\mu = \left(kT/f_e^2\right)(F + G)/2\mu_g \quad (15)$$

The dipole moment in the excited Franck-Condon state (μ_e^{FC}) was determined by the formula

$$\Delta^a\mu = \left(\mu_e^{FC} - \mu_g\right) \quad (16)$$

which is sufficiently valid for low-polar solvents. The dipole moments of flavonols obtained as an average over several independent EOAM measurements in different solutions are shown in Table III.

The amino-group in the side ring of 3-hydroxyflavone has a substantial influence on the π-electron density distribution in the ground state, and an even more stronger influence in the excited S_1-state [18–23]. From our results follows that the intramolecular H-bond between the hydroxy-group and carbonyl oxygen in the normal form (Fig. 1) also plays some role in the charge

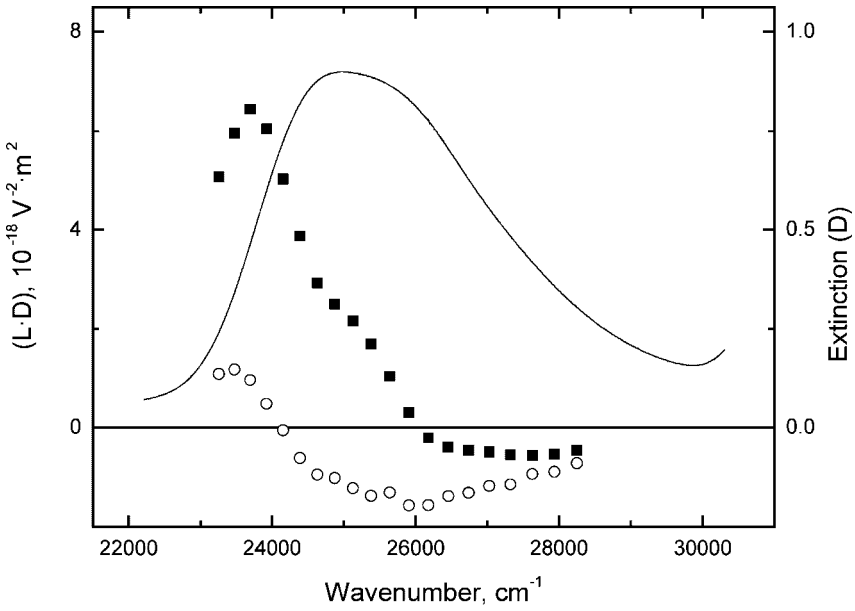

Fig. 5. Absorption (solid curve) and electrooptical absorption (■—$\chi = 0$, ○—$\chi = \pi/2$) spectra of FCR in 1,4-dioxane at $T = 298$ K.

distribution. Among the studied flavonols (see Table III), the value of the dipole moment μ_g is lower for FCR and FME than in the case of FME3ME in all solvents. This can be understood with the existence of an intramolecular H-bond in the case of FCR and FME, which partly compensates a negative charge on the carbonyl oxygen.

Nevertheless, the value of the change of the dipole moment vector after excitation to the Franck-Condon state $\Delta^a\mu$ for FCR and FME in different solvents is practically not solvent dependent. This means that the polarity of the solvent does not essentially influence on the efficiency of intramolecular charge transfer and therefore on the dipole moments of FCR and FME.

Table II. Electrooptical Coefficients Obtained by EOAM for FME in Toluene at $T = 298$ K

D (V^{-2} m^2)/10^{-20}	124.4 ± 9
E (V^{-2} m^2)/10^{-20}	5129.2 ± 177
F (CV^{-1} m^2)/10^{-40}	2782.5 ± 62.7
G (CV^{-1} m^2)/10^{-40}	2766.9 ± 63
H (C^2 m^2)/10^{-60}	3221 ± 187
I (C^2 m^2)/10^{-60}	3165.3 ± 232

Note. The coefficients and their standard deviations were obtained from fitting of the experimental L values by the program SYSTAT version 7.0 according to Eq. (2).

CONCLUSIONS

As follows from our experiments in the case of FCR, FME, and FME3ME the ground and excited Franck-Condon state electrical dipole moments are parallel to each other and also parallel to the transition dipole moment. The electrical dipole moments of the normal form of FCR, FME, and FME3ME in the ground state in different solvents have values within the range (12.0–17.7) $\times 10^{-30}$ C m. Upon optical excitation the dipole moments increase by (41.9–52.9) $\times 10^{-30}$ C m. This increase of the dipole moments is much larger than in the case of the parent 3-hydroxyflavone [11] and can be explained by intramolecular charge transfer from the amino group to the carbonyl group. Among the studied flavonols, μ_g of FME3ME is lager then those of FCR and FME. This effect can be explained by the existence of an intramolecular H-bond in the case of FCR and FME, which partly compensates a negative charge on the carbonyl oxygen in the ground state. The value of the change of the dipole moment vector after excitation to the Franck-Condon state $\Delta^a\mu$ and the value of the vector μ_e^{FC} for FCR and FME in different solvents is practically equal for every compound. Hence, FCR and FME are very perspective probes for the investigation of dielectric interactions in biological systems due to their large values of the dipole moments and the independence of these values on the polarity of the sites. Also, due to the large value of $\Delta^a\mu$ for FCR and FME the

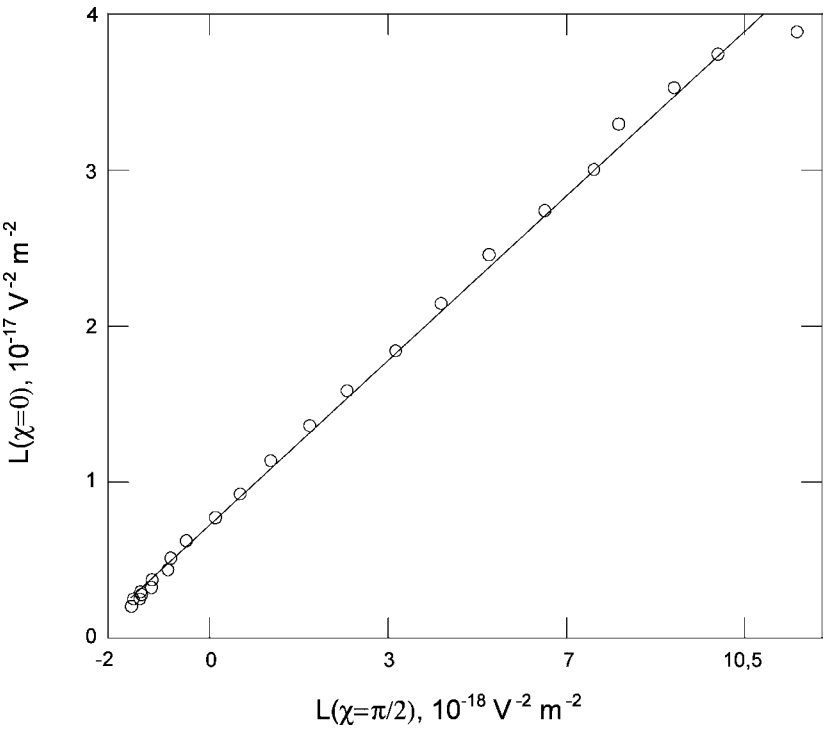

Fig. 6. Plot of $L(\nu, \chi = 0)\nu, \chi = 0)$ versus $L(\nu, \chi = \pi/2)\nu\chi = \pi/2)$ of FCR in 1,4-dioxane at $T = 298$ K. The points show the experimental data and the line is their approximation by a linear fit.

electronic spectra of these probes are inhomogeneously broadened, as is shown in Ref. 18. Hence, it is possible to excite selectively the probes located in different sites, and study the distribution of micropolarity in biological membranes. Additional information about dielectric interactions in the surrounding may be obtained from the ratio of the I_N^*/I_T^* fluorescence peak intensities of the normal form and phototautomer, which allows two-wavelength ratiometric detection in biological systems.

Table III. The Average Values of the Dipole Moments of FCR, FME, and FME3ME in Different Solvents at $T = 298$ K obtained from Several Independent Measurements (1 Debye $= 0.3 \times 10^{-30}$C m)

Molecule	μ_g/ (10^{-30}C m)	$\Delta^a\mu$/ (10^{-30}C m)	μ_e^{FC}/ (10^{-30}C m)
FCR in 1,4-dioxane	15.3 ± 0.4	47.1 ± 1.6	62.4 ± 1.2
FCR in toluene	16.1 ± 0.1	48.8 ± 0.1	64.9 ± 0.2
FCR in cyclohexane	15.1 ± 1.5	50.3 ± 2.3	65.4 ± 1.2
FME in 1,4-dioxane	14.1 ± 0.1	45.1 ± 0.95	59.2 ± 0.95
FME in toluene	15.6 ± 0.1	47.7 ± 0.03	63.3 ± 0.13
FME in cyclohexane	12.0 ± 0.2	52.3 ± 2.3	64.3 ± 2.0
FME3ME in 1,4-dioxane	17.7 ± 0.1	50.4 ± 1.6	68.1 ± 1.5
FME3ME in toluene	17.7 ± 0.1	52.9 ± 0.1	70.6 ± 0.2
FME3ME in cyclohexane	16.4 ± 0.3	41.9 ± 0.3	58.3 ± 0.6

Note. μ_g-the dipole moment in the ground state, $\Delta^a\mu$-the change of the dipole moment vector with excitation to the Franck-Condon state, μ_e^{FC}-the dipole moment in the excited Franck-Condon state.

ACKNOWLEDGMENTS

The authors thank Mr. A. S. Kozlovsky for writing the program of a decomposition of the fluorescence spectra on separate bands. This work was supported partly by the ISTC, project # B-479 and Byelarussian Republican Fund of Fundamental Research, project F01MS-009.

REFERENCES

1. J. R. Lakowicz (1999). *Principles of Fluorescent Spectroscopy*, 2nd ed., Plenum Press, New York.
2. J. R. Lakowicz (1983). *Principles of Fluorescent Spectroscopy*, Plenum Press, New York.
3. G. E. Dobretsov (1989). *Fluorescent Probes for Studying Cells, Membranes and Proteins*, Nauka, Moscow (in Russian).
4. H. Träuble and H. Eibl (1974). Electrostatic effects on lipid phase transitions: membrane structure and ionic environment. *Proc. Natl. Acad. Sci. USA* **71**, 214–219.

5. F. Bellemare and M. Fragata (1980). Polarity studies on the head group of single-layered phosphatidylcholine-α-tocopherol vesicles. *J. Coll. Interf. Sci.* **77**, 243–252.
6. M. Fragata and F. Bellemare (1985). Micropolarities of lipid bilayers and micelles. II. Application limits of dielectric constant determinations with the polarity probe α-tocopherol. *J. Coll. Interf. Sci.* **107**, 553–559.
7. Y. Kimura and A. Ikegami (1985). Local dielectric properties around polar region of lipid bilayer membranes. *J. Membrane Biol.* **85**, 225–231.
8. N. A. Nemkovich, A. N. Rubinov, and M. G. Savvidi (1992). Fluorescence decay studies of probe molecules distribution throughout the lipid membranes and the determination of 'micropolarity'. *Inst. Phys. Conf. Ser.* **126**, 639–642.
9. N. A. Nemkovich and A. N. Rubinov (1995). Spectral inhomogeneity and wavelength-dependent rotation of probe molecules in membranes. *J. Fluorescence* **5**, 285–294.
10. N. A. Nemkovich, W. Baumann, J. V. Kruchenok, H. Reis, and A. N. Rubinov (1997). Dipole moments of phenylnaphthylamine fluorescence probes and study of dielectric interactions in human erythrocyte ghosts. *J. Fluorescence* **7**, 363–370.
11. L. V. Premvardhan and L. A. Peteanu (1999). Dipolar properties of and temperature effects on the electronic states of 3-hydroxyflavone (3HF) determined using Stark-effect spectroscopy and compared to electronic structure calculations. *J. Phys. Chem. A* **103**, 7506–7514.
12. A. Chowdhury, L. Yu, I. Raheem, L. Peteanu, L. A. Liu, and D. J. Yaron (2003). Stark spectroscopy of size-selected helical H-aggregates of a cyanine dye templated by duplex DNA. Effect of exciton coupling on electronic polarizebilities. *J. Phys. Chem. A* **107**, 3351–3362.
13. W. Baumann (1989). In B. W. Rossiter and J. F. Hamitton (Eds.), *Physical Methods of Chemistry*, Vol. 3b, Wiley, Newe York, pp. 45–131.
14. W. Rettig and W. Baumann (1992). In J. F. Ralek (Ed.), *Progress in Photochemistry and Photophysics*, Vol. 6, CRC Press, Boca Raton, pp. 79–134.
15. N. A. Nemkovich, W. Baumann, H. Reis, and N. Detzer (1995). Dipole moments of aminophtalimides determined by modified electro-optical absorption and emission measurements. *J. Photochem. Photobiol. A: Chem.* **89**, 127–133.
16. N. A. Nemkovich, W. Baumann, and V. G. Pivovarenko (2002). Dipole moments of 4′-aminoflavonols using electro-optical absorption measurements or molecular Stark-effect spectroscopy. *J. Photochem. Photobiol. A: Chem.* **153**, 19–24.
17. N. A. Nemkovich, V. I. Tomin, and A. N. Rubinov (1991). In J. R. Lakowicz (Ed.), *Topic in Fluorescence Spectroscopy, Vol. 2: Principles*, Plenum Press, New York, pp. 367–428.
18. N. A. Nemkovich, J. V. Kruchenok, A. N. Rubinov, V. G. Pivovarenko, and W. Baumann (2001). Site selectivity in excited-state intramolecular proton transfer in flavonols. *J. Photochem. Photobiol. A: Chem.* **139**, 53–62.
19. P.-T. Chou, M. L. Martinez, and J.-H. Clements, (1993). Reversal of excitation behavior of proton-transfer vs. Charge-transfer by dielectric perturbation of electronic manifolds. *J. Phys. Chem.* **97**, 2618–2622.
20. S. M. Ormson, R. G. Brown, F. Vollmer, and W. Rettig (1994). Switching between charge- and proton-transfer emission in the excited state of a substituted 3-hydroxyflavone. *J. Photochem. Photobiol. A: Chem.* **81**, 65–72.
21. A. D. Roshal, A. V. Grigorovich, A. O. Doroshenko, V. G. Pivovarenko, and A. P. Demchenko (1998). Flavonols and crown-flavonols as metal cation chelators. The different nature of Ba^{2+} and Mg^{2+} complexes. *J. Phys. Chem.* **102**, 5907–5914.
22. A. S. Klymchenko and A. P. Demchenko (2003). Multiparametric probing of intermolecular interactions with fluorescent dye exhibiting intramolecular proton transfer. *Phys. Chem. Chem. Phys.* **5**, 461–468.
23. A. D. Roshal, J. A. Organero, and A. Douhal (2003). Tuning the mechanism of proton-transfer in a hydroxyflavone derivative. *Chem. Phys. Lett.* **379**, 53–59.
24. M. A. Smith, R. M. Neumann, and R. A. Webb (1968). A modification of the Algar-Flynn-Oyamada preparation of flavonols. *J. Heterocyclic Chem.* **5**, 425–426.
25. E. Liptay (1974). In E. C. Lim (Ed.), *Excited States*, Vol. 1, Academic press, New York, pp. 129–229.
26. N. G. Bakhshiev (1989). *Solvatochromy: Problems and Methods*, Leningrad University Press, Leningrad (in Russian).
27. W. Baumann, Z. Nagy, H. Reis, and N. Detzer (1994). Electric field-induced anisotropy spectra. *Chem. Phys. Lett.* **224**, 517–524.
28. H. Reis (1995). *Dissertation zur Erlangung des Grades "Doktor der Naturwissenschaften" am Fachbereich Chemie und Pharmazie der Johhannes Gutenberg-Universitat in Mainz*, Mainz.