

Excitation Energy Transport in a Concentrated System of Flavomononucleotide in Polyvinyl Alcohol Films

Piotr Bojarski,¹ Hanna Grajek,² Grażyna Żurkowska,³ Benedykt Kukliński,¹
Bogdan Smyk,² and Regina Drabent²

Received August 7, 1998; accepted April 20, 1999

Excitation energy transport mechanism of flavomononucleotide polyvinyl alcohol films is studied. Excitation wavelength and temperature dependences of fluorescence spectra and quantum yields for different concentrations of the dye are investigated. These measurements together with those of absorption reveal that dimers are imperfect traps for excitation energy and that the energy transfer can occur both in the forward and in the reverse direction. It was found that, contrary to liquid solutions, the dimerization constant of flavomononucleotide in polyvinyl alcohol films does not depend on temperature and that the irregularities observed can be explained by the temperature-dependent changes in the quantum yield of the monomer-dimer system and the effect of inhomogeneous orientation broadening of energy levels.

KEY WORDS: Flavomononucleotide; energy transfer; absorption; dimers; inhomogeneous broadening.

INTRODUCTION

Flavins as photoreceptors and coenzymes take part in numerous important biological processes [1]. They absorb light in the ultraviolet as well as in the visible region, where they emit fluorescence with a quantum yield of 0.26 (in H₂O). Previous studies [2,3] have shown that the spectroscopic properties of flavins (FMN) change at high concentrations in aqueous solutions, since they form dimers which play a role of perfect traps for the excitation energy [3]. Recently, it has been shown, however, that FMN dimers [4] as well as dimers of some other dyes, i.e., rhodamines, can be fluorescent in rigid systems. In this report we studied the concentration

behavior of FMN in polyvinyl alcohol (PVA) films for different temperatures and excitation wavelengths.

Recently, the measurements of absorption and fluorescence spectra of flavomononucleotide (FMN) in PVA films were carried out by us over a very wide concentration range [5]. Temperature, excitation wavelength, and concentration studies of FMN in PVA allowed us to explain qualitatively the regularities observed. At very high concentrations FMN dimers are fluorescent and they play the role of imperfect traps for excitation energy. The monomers excited by light absorption can transfer nonradiatively the excitation energy to other unexcited monomers in one or many steps (energy migration) with the rate constant k_{MM} as well as to dimers with the rate constant k_{MD} . Since the fluorescence spectrum of dimers overlaps partially with that of monomer absorption, not only forward but also reverse energy transfer from dimers to monomers is possible [6]. For the recorded fluorescence spectrum of such concentrated systems, both monomers and dimers are responsible. This spectrum is the sum of fluorescence emitted by monomers and dimers

¹ University of Gdańsk, Institute of Experimental Physics, Luminescence Research Group, Wita Stwosza 57, 80-952 Gdańsk, Poland.

² Department of Physics and Biophysics, University of Agriculture, 10-957, Olsztyn, Poland.

³ Department of Technical Physics and Applied Mathematics, Technical University of Gdańsk, Narutowicza 11/12, Poland.

excited directly by light absorption as well as by forward and reverse energy transfer. Quantitative interpretation of the concentration and temperature changes observed in the fluorescence spectra requires the knowledge of critical distances for forward transfer, R_0^{MM} and R_0^{MD} , as well as for reverse transfer, R_0^{DM} and R_0^{DD} . Determination of R_0^{MM} and R_0^{MD} is not difficult, whereas the unknown dimer fluorescence does not allow calculation of the critical distances R_0^{DM} and R_0^{DD} and dimer quantum yield [7].

An additional problem in the interpretation of the above-mentioned spectra is the effect of inhomogeneous broadening of energy levels of FMN molecules, which is clearly seen in the system of interest. This effect in the concentrated systems leads to directional energy transfer from "blue" to "red" centers [8]. To obtain additional information on the FMN properties in PVA films, measurements of absorption and fluorescence spectra at a lower temperature ($T = 263$ K) were performed. At this temperature an increased effect of the dimer's presence on the fluorescence spectra is expected. Also, measurements of the absolute quantum yield of FMN in PVA over a wide concentration range and for temperatures from 263 to 323 K were carried out. The relative temperature changes of the quantum yield obtained experimentally are compared with the results of the hopping model assuming that in the system investigated only forward energy transport between monomers and from monomers to dimers takes place.

EXPERIMENTAL

Flavomononucleotide FMN [riboflavin-5'-monophosphate, sodium salt FMN; Na ($C_{13}H_{20}N_4NaO_9P \cdot 2H_2O$), 97% pure, from Fluka AG and polyvinyl alcohol from Loba-Chemie, Vien-Fishamend, were used. FM was not additionally purified. FMN was dissolved in a 10% aqueous solution of PVA at a temperature of about 350 K to obtain a homogeneous solution. The FMN concentration C in PVA films changed from 6.9×10^{-4} to 6.8×10^{-1} M. The samples of concentrations $C > 10^{-2}$ M were obtained by drawing out the glass plates from the solution and evaporation. This method allows us to obtain samples of such a thickness d that relation (1) is fulfilled. Samples of concentrations $C < 10^{-2}$ M were obtained by carefully pouring a small amount of the solution on horizontally placed polished glass plates and evaporation. The thickness d of the sample was adjusted in each case so that the following relation was valid [9]:

$$2.3\epsilon^{\max} Cd < 0.1 \quad (1)$$

where ϵ^{\max} is the maximum value of the monomer extinc-

tion coefficient. Under this condition, the influence of secondary effects on the fluorescence observables may be neglected.

Absorption spectra were measured using a Varian Cary 3E spectrometer. The fluorescence spectra and quantum yields were measured upon the front-face excitation, with observation of the sample using the apparatus described separately [10,11], and the results were corrected for the spectral sensitivity of the equipment as well as for the spatial anisotropy distribution of the polarized fluorescence [12,13]. The spectra and quantum yields were measured over the whole range of FMN concentrations at the excitation wavelengths 445 and 490 nm at 10 K intervals from 263 to 323 K. The temperature of each measurement was controlled with an accuracy of $\pm 0.1^\circ$.

RESULTS AND DISCUSSION

Figure 1 shows an example of absorption spectra of FMN in PVA at $T = 263$ K and $T = 323$ K for a high concentration $C = 0.17$ M. As shown, the extinction coefficients differ slightly for different temperatures. At other concentrations this difference is comparably small. Such a weak dependence of absorption spectra on temperature indicates that also the dimerization constant ($K = 11.6$ M [5]) does not, in this case, depend on the temperature. Hence, it can be concluded that the temperature changes of fluorescence spectra should be connected mainly with the change in the absolute quantum yield of monomers and dimers (independently of the temperature effect on nonradiative energy transport parameters). Figure 2 shows the fluorescence spectra of FMN in PVA for systems of lower (Fig. 2A) and higher (Fig. 2B) concentrations for several temperatures at an excitation $\lambda_{exc} = 445$ nm, corresponding to the maximum of monomer absorption. It should be emphasized that for $C = 0.68$ M the concentration of dimers exceeds that of monomers ($C_M = 1.51 \times 10^{-1}$ M and $C_D = 2.64 \times 10^{-1}$ M, calculated for the equilibrium constant $K = 11.6$ M $^{-1}$ at $T = 293$ K [5]), whereas for the lower concentration the concentration of dimers is about three times lower than that of monomers. The location of peaks ($\lambda_{max} = 518$ nm at $T = 263$ K; Fig. 2A) changes only slightly with temperature. However, for the concentrated system the peak location is red shifted almost 30 nm and the half-widths of the bands are distinctly larger than for the system of a lower concentration (Fig. 2B). The effect of red shift may be explained by inhomogeneous orientation broadening of FMN energy levels (IOBEL) in the PVA matrix and by the presence of fluorescing dimers.

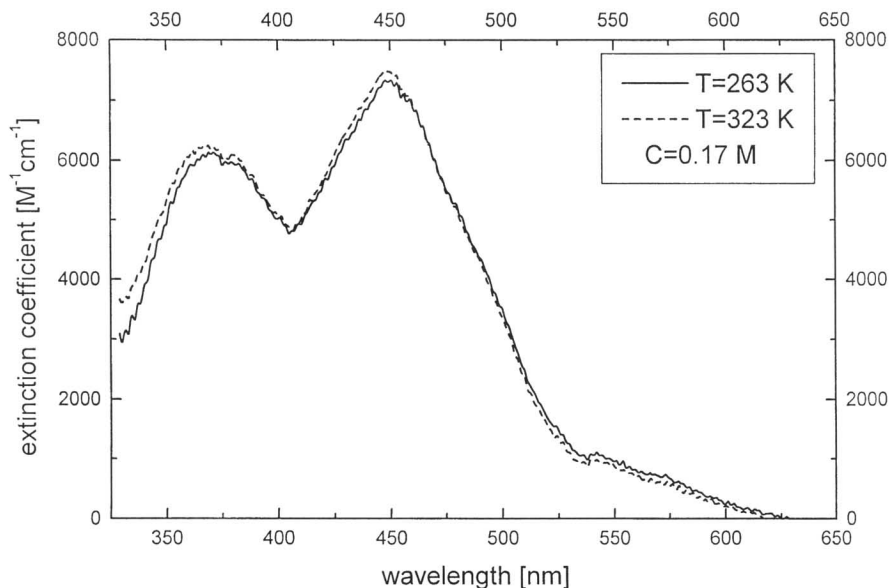


Fig. 1. Absorption spectra of a ($C = 0.17 M$) concentrated FMN sample at different temperatures.

Figure 3 shows the fluorescence spectra of the same systems for long-wavelength excitation, $\lambda_{\text{exc}} = 490 \text{ nm}$. This time the peak locations of fluorescence spectra for lower and higher concentrations are close and they amount to $\sim 548 \text{ nm}$, whereas the half-widths of fluorescence spectra are distinctly smaller for systems of lower (Figs. 2A and 3A, Table I) than higher (Figs. 2B and 3B, Table I) concentrations. The long-wavelength excitation may be absorbed only by fractions of monomers with the lowest values of electronic transition energies E_{0M} . Excitation transfer back to the monomer group of higher energy E_{0M} is not very probable. The same concerns the system of high concentration, with the difference that the excitation energy may be transferred to fluorescent dimers. Hence, the half-width of concentrated systems is larger. Let us note that at the excitation $\lambda_{\text{exc}} = 445 \text{ nm}$ the half-widths are distinctly larger for both lower and higher concentrations. Table I lists the values of the quantum yield (for several concentrations and two temperatures), wavelength of the fluorescence peak, and half-width of the band. Only a quantum yield corresponding to $C = 6.9 \times 10^{-4} M$ (given in the second column in Table I) can be treated as the absolute quantum yield of a monomer. For higher concentrations of FMN molecules the values listed are the quantum yield of a monomer-dimer system in the presence of forward and reverse excitation energy transfer. Relative values of the quantum yield, η/η_0 , are compared with those obtained from the hopping model, in which only forward energy transport from monomers to monomers and from monomers to dimers is taken into account. The theoretical values of

η/η_0 were calculated from the following expression [14] for the values of critical concentrations and absolute quantum yield of monomers given in Table I:

$$\eta_M/\eta_{0M} = \frac{1 - f(\gamma)}{1 - \alpha f(\gamma)} \quad (2)$$

where η_{0M} is the monomer quantum yield measured in the absence of energy traps, and γ is the so-called reduced concentration given by

$$\gamma = \gamma_M + \gamma_D = \frac{\sqrt{\pi}}{2} \left(\frac{C_M}{\sqrt{2}C_0^{MM}} + \frac{C_D}{C_0^{MD}} \right) \quad (3)$$

where $f(\gamma)$ is related to the erf(γ) error function by

$$f(\gamma) = \sqrt{\pi}\gamma \exp(\gamma^2)(1 - \text{erf}(\gamma)) \quad (4)$$

and α is defined as

$$\alpha = \frac{\gamma_M/\sqrt{2}}{(\gamma_M/\sqrt{2}) + \gamma_D} \quad (5)$$

The critical concentrations C_0^{MX} , where $x \in \{M, D\}$, were determined from the relation [15]

$$C_0^{MX} = 4.23 \cdot 10^{-10} n^2 (\langle \kappa^2 \rangle \eta_{0M} I_{MX})^{-1/2} \quad (6)$$

where

$$I_{MX} = \int_0^\infty f_M(\nu) \epsilon_X(\nu) \nu^{-4} d\nu \quad (7)$$

is the overlap integral of the fluorescence spectral distribution $f_M(\nu)$ of a monomer molecule, expressed as the number of quanta and normalized to unity with the

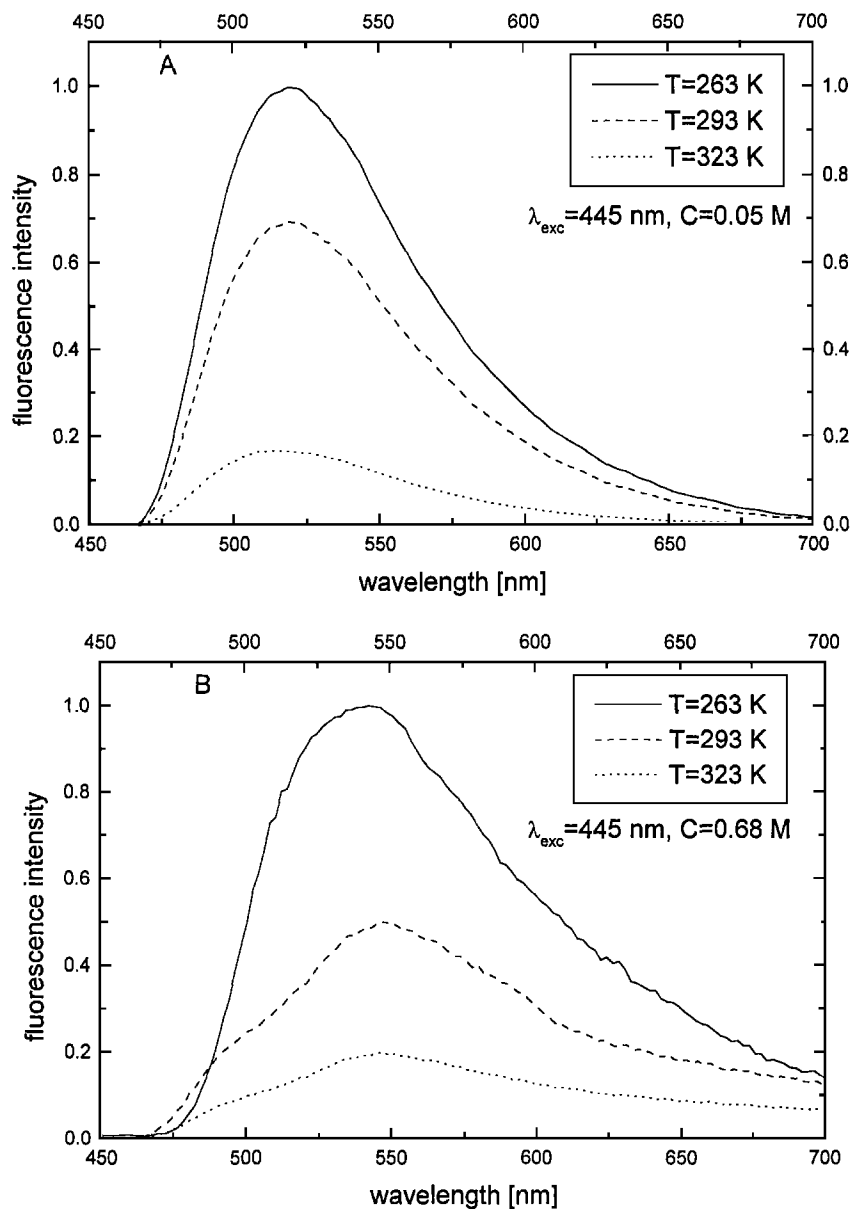


Fig. 2. Temperature effect on fluorescence spectra of FMN in PVA at $\lambda_{\text{exc}} = 445$ nm for (A) a lower concentration, $C = 0.05$ M, and (B) a higher concentration, $C = 0.68$ M.

absorption spectrum $\epsilon_X(\nu)$ of molecule X , n denotes the refractive index of the medium, ν is the wavenumber, and $\langle \kappa^2 \rangle$ is the averaged orientation factor.

Theoretical values, η/η_0 , were calculated for the critical concentrations given in Table I and corresponding to the values of quantum yield η_0 specified therein. These values were determined experimentally for particular temperatures. As shown in Table I the experimental values of η/η_0 are distinctly higher than those calculated. This regularity indicates that, to describe the quoted experimental values quantitatively, a theory accounting for the reverse energy transport from dimers to monomers

is indispensable. This is because its contribution increases with concentration and decreases with temperature (since the absolute quantum yield also decreases with temperature). As a result, at a high temperature dimers should become perfect traps for excitation energy and reverse transport does not occur.

CONCLUSIONS

Excitation energy transport in a monomer–dimer system of FMN in PVA films was studied. It was found

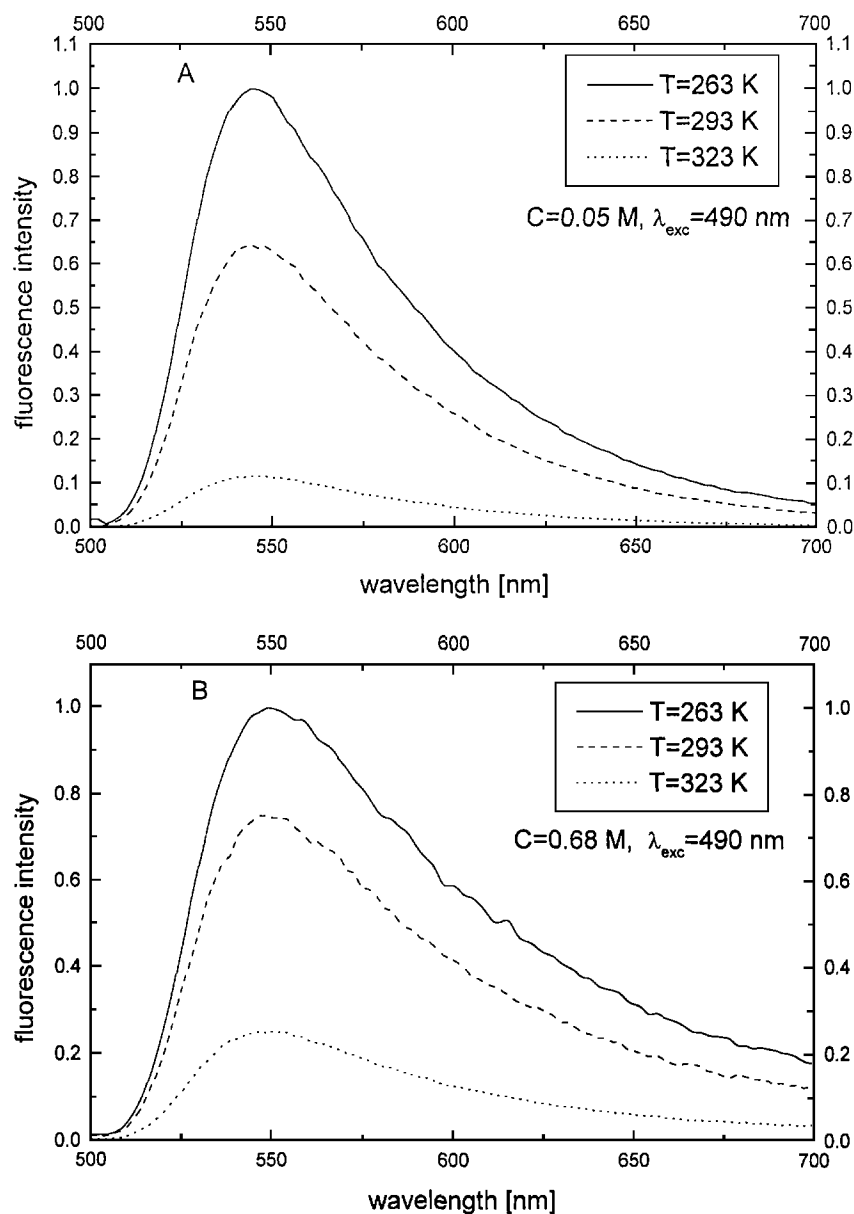


Fig. 3. Temperature effect on fluorescence spectra of FMN in PVA at $\lambda_{\text{exc}} = 490$ nm for (A) a lower concentration, $C = 0.05$ M, and (B) a higher concentration, $C = 0.68$ M.

that the mechanism of this transport is very complex since it depends on the excitation wavelength and temperature. The dispersive character of energy transport in this system is due to inhomogeneous orientation broadening of energy levels, which manifests itself in the red shift of fluorescence spectra with concentration at shorter excitation of the system (445 nm). As expected, this effect decreases with temperature for shorter excitation and it seems not to appear when the system is excited at 490 nm over the concentration range studied, irrespective of the temperature.

A comparison of the experimental relative quantum yield with the hopping theory of forward energy transport showed that the measured values are higher than those predicted by the theory. Dimer fluorescence at high concentrations and the reverse transfer from them to monomers are responsible for this. Direct comparison to theories accounting for the latter effect is difficult since it requires that the dimer quantum yield and its pure fluorescence spectrum be known. However, the dimerization constant of FMN in PVA (rigid system) does not seem to depend on temperature within the range studied

Table I. Selected Spectroscopic Data and Energy Transfer Parameters for FMN in PVA at Different Temperatures, Excitation Wavelengths, and Concentrations

	$C(M)$	η (experiment) $\pm 10\%$	η/η_0 (experiment) $\pm 10\%$	η/η_0 , hopping theory	λ_{\max} (nm)	$\Delta\lambda_{1/2}$ (nm)
$\lambda = 445 \text{ nm}, T = 263 \text{ K}, \eta_0 = 0.65$ (measured for $C = 0.00069 \text{ M}, C_{0MM} = 0.04 \text{ M}, C_{0MD} = 0.025 \text{ M}$)	0.05	0.34	0.52	0.4915	518	83
	0.17	0.077	0.12	0.0792	526	97
	0.27	0.026	0.04	0.0313	529	102
	0.68	0.02	0.03	0.004	543	109
$\lambda = 445 \text{ nm}, T = 323 \text{ K}, \eta_0 = 0.07$ (measured for $C = 0.00069 \text{ M}, C_{0MM} = 0.12 \text{ M}, C_{0MD} = 0.08 \text{ M}$)	0.05	0.057	0.81	0.809	519	82
	0.17	0.028	0.4	0.367	518	95
	0.27	0.017	0.24	0.204	523	99
	0.68	0.004	0.06	0.04	545	124
$\lambda = 490 \text{ nm}, T = 263 \text{ K}, \eta_0 = 0.41$ (measured for $C = 0.00069 \text{ M}, C_{0MM} = 0.07 \text{ M}, C_{0MD} = 0.05 \text{ M}$)	0.05	0.31	0.75	0.704	548	65
	0.17	0.11	0.27	0.217	546	75
	0.27	0.06	0.15	0.102	546	76
	0.68	0.02	0.05	0.017	548	85
$\lambda = 490 \text{ nm}, T = 323 \text{ K}, \eta_0 = 0.04$ (measured for $C = 0.00069 \text{ M}, C_{0MM} = 0.22 \text{ M}, C_{0MD} = 0.16 \text{ M}$)	0.05	0.036	0.9	0.901	548	66
	0.17	0.024	0.6	0.596	543	71
	0.27	0.017	0.43	0.419	548	72
	0.68	0.005	0.12	0.126	550	74

and therefore the differences observed in the fluorescence spectra of concentrated solutions can be associated mainly with the change in dimer quantum yield. We plan to use this fact in further investigations to estimate the dimer quantum yield.

ACKNOWLEDGMENTS

This paper is dedicated to Prof. Dr. C. Bojarski on the occasion of his 75th birthday. We would like to thank the DAAD for the granted photomultiplier. This work was supported by Grants BW 03030.802, 03030.215, and 5200-5-0278-9.

REFERENCES

1. W. Haupt and G. Wagner (1984) in G. Colombetti and F. Lenci (Eds.), *Membranes and Sensory Transduction*, Plenum Press, New York, pp. 331–375.
2. H. Grajek, G. Żurkowska, R. Drabent, and C. Bojarski (1986) *Biochim. Biophys. Acta* **881**, 241–247.
3. H. Grajek, C. Bojarski, G. Żurkowska, and R. Drabent (1992) *Photochem. Photobiol.* **55**, 381–387.
4. G. Żurkowska, H. Grajek, and C. Bojarski (1996) *Curr. Topics Biophys.* **20**, 134–138.
5. H. Grajek, G. Żurkowska, P. Bojarski, B. Kukliński, B. Smyk, R. Drabent, and C. Bojarski (1998) *Biochim. Biophys. Acta* **184**, 253–267.
6. C. Bojarski (1984) *Z. Naturforsch.* **39a**, 948–951.
7. P. Bojarski, A. Matczuk, C. Bojarski, A. Kawski, B. Kukliński, G. Żurkowska, and H. Diehl (1996) *Chem. Phys.* **210**, 485–499.
8. A. N. Rubinov, V. I. Tomin, and B. A. Bushuk (1982) *J. Luminesc.* **26**, 367–376.
9. I. Ketskemety, J. Dombi, R. Horvai, J. Hevesi and L. Kozma, *Acta Phys. Chem. (Szeged)* (1961) **7**, 17–21.
10. B. Smyk and R. Drabent (1989) *Analyst* **114**, 723–726.
11. A. Kawski and K. Nowaczyk (1990) *Acta Phys. Polon.* **A78**, 379–392.
12. P. P. Feofilov (1961) *The Physical Basis of Polarized Emission* (English translation), Consultant Bureau, New York.
13. R. E. Dale and R. K. Bauer (1971) *Acta Phys. Polon.* **A40**, 853–882.
14. R. Twardowski and C. Bojarski (1985) *J. Luminesc.* **33**, 79–85.
15. Th. Förster (1948) *Ann. Phys.* **2**, 55–75.