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Mirror Symmetry of Absorption and Fluorescence Spectra of Chlorophyll a Solutions Measured at Room Temperature

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The mirror symmetry of the fluorescence spectrum of chlorophyll a in diluted solutions ($C \sim 10^{-6}$ M/l) relative to the absorption spectrum was experimentally demonstrated by measurements of these spectra at the room temperatures. The measurements were taken with thirteen organic solvents of different physical parameters — refractive index and dielectric constant on the half-width of both principal spectral bands was demonstrated. The magnitude of Stokes shift of fluorescence maxima is strongly affected by refractive index of the solvent.

Зеркальная симметрия спектра флуоресценции хлорофилла a в отношении к спектру поглощения в разбавленных растворах ($C \sim 10^{-6}$ M/l) была проверена экспериментально измерениями этих спектров при комнатной температуре. Измерения совершены в тринадцати органических растворителях имеющих разные физические свойства-показатель преломления n и диэлектрическую постоянную D. Было показано влияние показателя преломления и диэлектрической постоянной на полуширину обеих главных спектральных полось. Показатель преломления растворителя строго влияет на величину сдвига Стоукса максимума флуоресценции.

Experimentálně byla ověřena zrcadlová symetrie fluorescenčního spektra chlorofylu a ve zředěných roztocích ($C \sim 10^{-6}$ M/l) vůči absorpčnímu spektru měřeními těchto spekter při pokojové teplotě. Měření byla provedena se 13 organickými rozpouštědly o různých fyzikálních vlastnostech — indexu lomu n a dielektrické konstanty D. Z měření je patrný vliv indexu lomu a dielektrické konstanty na pološířku obou hlavních spektrálních pásů. Velikost Stokesova posuvu fluorescenčních maxim se silně mění s indexem lomu rozpustidla.

I. Experimental

Chromatographically pure chlorophyll a was prepared in the Departement of Chemical Physics of the Charles University in Prague by the method described in detail in [1]. The preparation of diluted solutions of chlorophyll a with concentra-

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tions $\sim 10^{-6}$ M/l and measurements of their absorption and fluorescence spectra were done under conditions minimizing the possibility of photooxydation of chlorophyll a (inert atmosphere, closed cuvette). The solvents were R.P. grade and except for acetone, benzene, ethanol and methanol, were used without being further purified. The concentrations of solutions were determined from the absorption spectra with application of molar extinction coefficients which were determined by Seely in [2]. The absorption spectra were measured on the spectrophotometer SPECORD Zeiss (spectral slit width 30 cm^{-1}). For measurements of fluorescence we used spectrophotometer [3] with monochromator SPM 2 Zeiss (grating 650/mm), a cooled M 13 photomultiplier (cathode type S-20) and a G 1 B 1 recorder. The fluorescence was excited in the region of the Soret band. The fluorescence spectra were corrected for the spectral dependence of photomultiplier sensitivity. The fluorescence spectra were measured with spectral slit width 0.8 nm. The evaluation of the experimental results was made by means of the ODRA 1013 computer.

2. Results and Discussion

Measurements of absorption and fluorescence spectra of diluted chlorophyll a solutions with concentrations $2 \cdot 10^{-6} - 12 \cdot 10^{-6}$ M/l at room temperature demonstrated the mirror symmetry of these spectra in all measured solvents. The typical absorption (A) and fluorescence (F) curves of some measured solutions and the mirrored reflection of fluorescence (F') with the axis of symmetry o passing through the point of intersection of both spectra are seen in Fig. 1. When considering these spectra we can see difference in course of the mirror symmetry in the region of the

Table 1. The parameters characterizing the mirror symmetry of absorption and fluorescence spectra of chlorophyll a in solutions

Solvent		$n_{20°C}$	$D_{20°C}$	δ_A [cm^{-1}]	δ_F [cm^{-1}]	I_{1A}/I_{2A}	I_{1F}/I_{2F}	$\Delta\nu_1$ [cm^{-1}]
methanol	me	1,3288	32,63	265	273	4,35	4,46	215,4
ether	ete	1,3497	9,34	200	225	5,85	5,87	179,4
acetone	ac	1,3591	20,7	215	235	5,25	6,48	184,9
ethanol	et	1,3610	24,3	240	270	4,07	4,63	207,9
n-hexane	nh	1,3749	1,89	180	215	6,32	7,37	73,4
i-propanol	ip	1,3776	18,3	230	245	4,48	4,77	192,77
n-propanol	np	1,3854	20,1	240	225	4,53	5,65	148,5
n-butanol	nbu	1,3991	17,1	234	227	4,50	5,613	153,08
triethylamine	te	1,4003	2,42	215	220	5,72	7,21	123,06
dioxane	dx	1,4221	2,209	210	230	5,97	5,99	126,2
cyclohexane	cy	1,4266	2,015	225	230	5,76	5,81	136,5
benzene	bz	1,5011	2,274	210	240	6,28	6,67	163,1
nitrobenzene	nb	1,5524	34,82	240	245	4,98	3,81	232,72

secondary bands (blue shift of the second absorption maximum relative to the mirror symmetric image F' of the corresponding fluorescence band in most solvents). The basic parameters characterizing the quasi-mirror symmetry of spectra of chlorophyll a are summarized in Table 1., where $\Delta_{\lambda 1}$ is the spectral difference of peaks of principal fluorescence and absorption bands, δ_F and δ_A are half-widths of these bands I_{1A}/I_{2A} and I_{1F}/I_{2F} are intensity ratios of both absorption and fluorescence bands respectively.
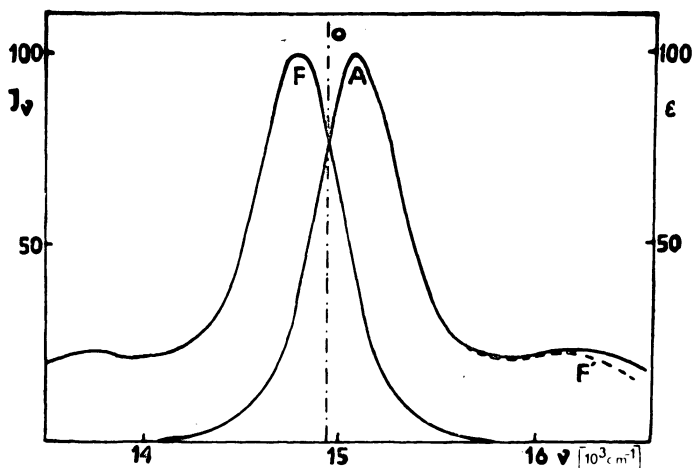

Fig. 1. The mirror symmetry of absorption and fluorescence spectra of chlorophyll a
in methanol

The half-width of the main absorption and fluorescence band depends essentially on the refractivity index (n) and dielectric constant (D) of the solvents, as illustrated in Fig. 2. The parameters of linear regression function

$$\delta = A \frac{D-1}{D+2} - \frac{n^2-1}{n^2+2} + B$$

were found by means of the method of least squares. For half-width of the absorption band the coefficients are $A = 303.45\,\text{cm}^{-1}$, $B = 47.13\,\text{cm}^{-1}$ and the correlation coefficient ϱ of linear regression function equals 0.8206, for fluorescence band $A = 263.95\,\text{cm}^{-1}$, $B = 81.12\,\text{cm}^{-1}$ and $\varrho = 0.9316$. This linear approximation was carried out for polar solvents and nitrobenzene. The values of δ_A, δ_F for non-polar solvents with low dielectric constant (n-hexane, triethylamine, dioxane, cyclohexane and benzene) do not fit in this approximation (Fig. 2).

In Tab. 1 the values of $\Delta_{\lambda 1}$ of Stokes shift of the main fluorescence band are also given. The magnitude of this shift depends on the physical parameters of solvents. In Fig. 3 the influence of the refractivity index of solvents on spectral difference of peaks of the main bands is illustrated.

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From the above experimental results and other measurements some conclusions can be drawn as for the spectral properties of chlorophyll a in solutions. The fluorescence spectrum of chlorophyll a being composed of two emission bands (the main one with $\lambda_{max} \sim 670$ nm, the secondary one with $\lambda_{max} \sim 720$ nm) is mirror symmetrical with respect to the absorption spectrum belonging to the first electron transition of chlorophyll a to the red band with $\lambda_{max} \sim 660$ nm and its vibrational satellite $\lambda_{max} \sim 630$ nm). The chlorophyll a in solutions $\sim 10^{-6}$ M/l keeps this
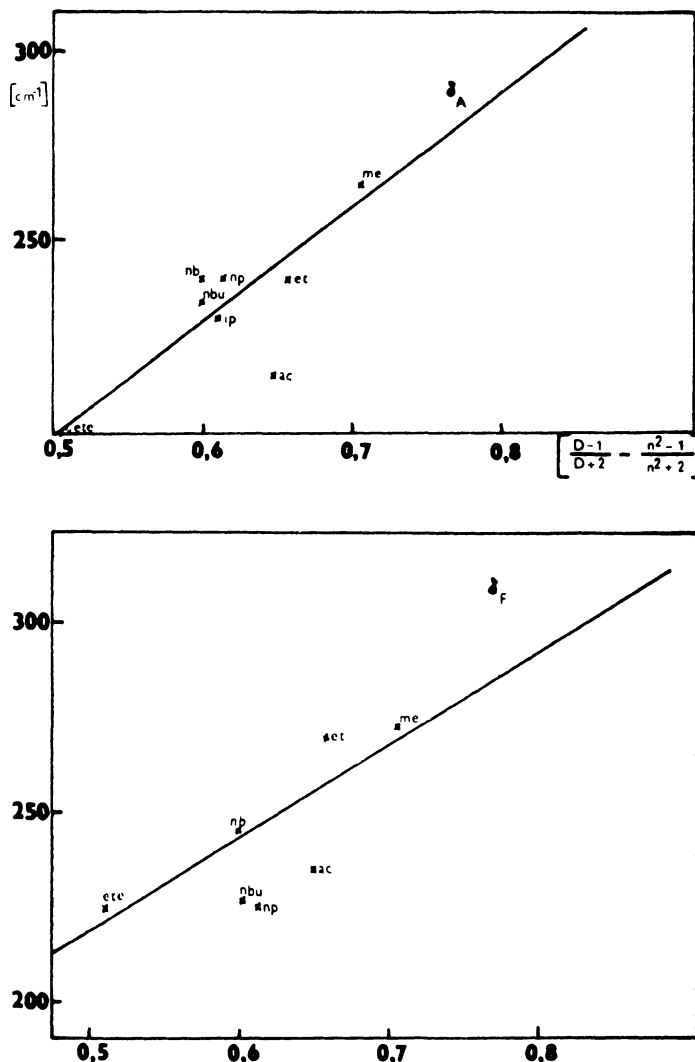

Fig. 2. The dependence of half-width of red absorption (δ_A) and flurescence (δ_F) bands on dielectric constant and refractivity index of solvents

property in all measured solvents with dielectric constants in the interval 1.89—34.82 and refractivity index in the interval 1.3288—1.5524. The differences in the intensity and in the position of the maximum of mirror symmetrical image of fluorescence F' and second absorption band (see Fig. 1), can be found either in the overlapping of this band and absorption originated from the second electron transition of chlorophyll a or in the composed structure of the long-wave emission band (see our paper [6]). An expressive broadening of the principal absorption and fluorescence bands
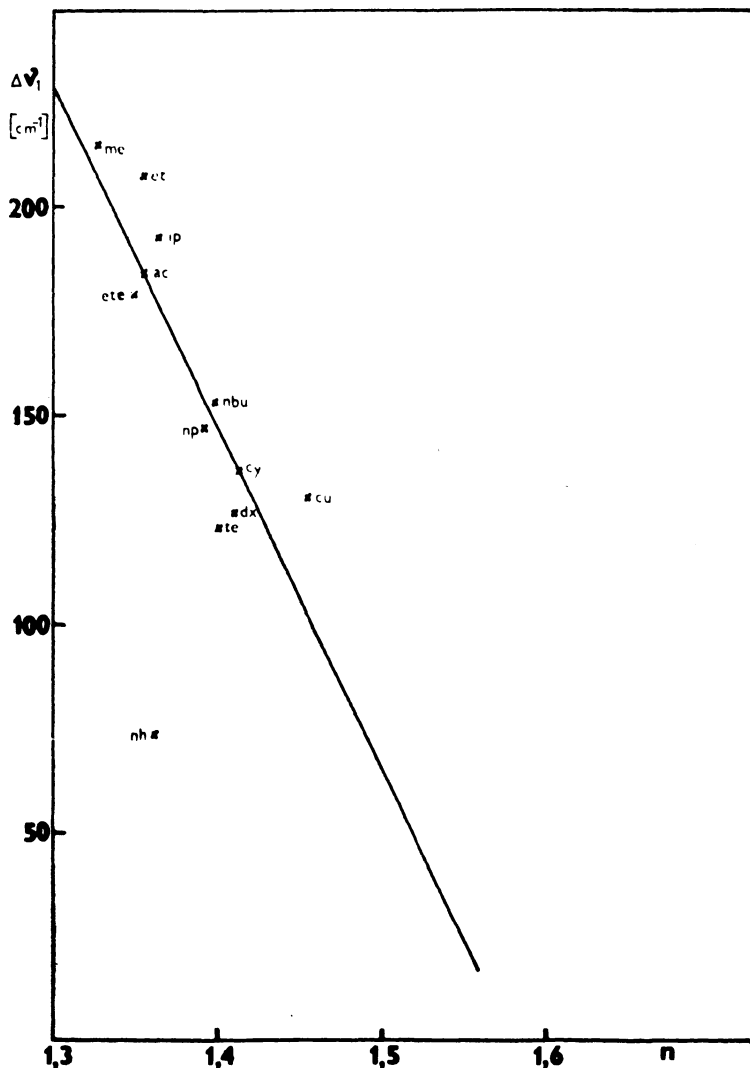

Fig. 3. The dependence of the spectral difference of maxima of the main absorption and fluorescence bands of chlorphyll a on the refractivity index of solvent

with increasing refractive index of solvents, independent on the polarity of the solvent, appears in a decrease of Stokes shift of the spectral maxima. The chlorophyll a in polar solvents with concentrations 10^{-5}—10^{-6} M/l exists in solvated form (P-solvate [7], [8]). In non-polar non-dried solvents the solvatation is realized by the assistance of the water molecules, which are present in sufficient amount in solutions with concentration 10^{-5}—10^{-6} M/l under usual conditions (N-solvate).

As demonstrated in our measurements, these two types of chlorophyll a solvates exhibit following spectral properties:

a) half-width of bands δ_{1A}, δ_{1F} of P-solvate of chlorophyll a is greater than these of N-solvate;

b) $\delta_A < \delta_F$ for P-solvate and for N-solvate is $\delta_A \sim \delta_F$;

c) the intensity ratios I_{1A}/I_{2A} and I_{1F}/I_{2F} are smaller for P-solvate than for N-solvate;

d) Stokes shift of the fluorescence spectrum of P-solvate is greater than that of N-solvate.

Further, more extensive studies will be published.

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