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Contribution to the Fluorescence of Chlorophyll a.

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1. Introduction

Recently several studies have been done of the luminescent properties of chlorophyll a in different media [1], [2], [3], [4], [5], [6]. The mesurements were performed with solutions of chlorophyll a in solvents of various polarity. The published results differ considerably and so no any unambiguous conclusions can be made of them. The reason of this situation is that for the experimental conditions being the results are affected by many factors as for instance the condition and purity of the dye which can itself decompose during experiment, the purity of solvent particularly the influence of polar impurities in non polar solvents, the effect of air oxygen etc. There is at present the generally accepted opinion that the absorption spectrum of porphyrins is formed only by the π-π^* electron transitions and it seems well experimentally supported [7], [8]. We can assume this in case of chlorophyll a too. It was concluded from the mirror symmetry of the two fluorescence bands of chlorophyll a (e.g. in acetone the shortwave band $\lambda_{max} \sim 670$ nm, the longwave band $\lambda_{max} \sim 720$ nm) with respect to the two longwave absorption bands ($\lambda_{max} \sim 615$, 662 nm) that the longwave fluorescence band is a second vibronic band of the same electronic transition as the shortwave fluorescence band. However it was pointed out that the mirror symmetry appeared only in suitable experimental conditions and the ratio of the intensities of both fluorescence bands varies very much as a function of temperature and chlorophyll concentration. Therefore the matter is more complicated and the character of the longwave fluorescence band is for the time being not explained unambiguously. We performed several measurements in our laboratory that could contribute to the explanation of problems connected with the properties of this longwave fluorescence band.

2. Results

We have prepared standard chlorophyll a solutions samples [9] and studied several fluorescence properties on them. The samples were chromatographically pure without any traces of pheophytin as well as the accompanying yellow dyes. The ratio of the maxima of absorption bands was $I_{430} : I_{662} = 1.32$ (in acetone).

There were performed two series of measurements of excitation spectra on the

benzene and acetone solution of chlorophyll a at the room temperature and one serie of measurements of the spectral course of fluorescence excited by He-Ne LASER on the 3-methyl pentane solution of the chlorophyll a at the room as well as liquid nitrogen temperature (experimental arrangement described before [10], [11], [12]).

The excitation spectra of chlorophyll a in benzene solution ($c = 1.3 \cdot 10^{-5}$ M) point out that the position of both fluorescence bands does not depend on the excitation wavelength. The comparison with the absorption spectrum shows good agreement in the whole studied region (400–650 nm). The relative variation of the intensity of both fluorescence bands $\frac{I_1 - I_2}{I_1 + I_2}$ (I_1 is the shortwave band) is constant within the limits of error through the whole region up to 650 nm (see fig. 1.). At the room temperature the mirror symmetry of both fluorescence bands with respect to the two longwave absorption bands remains valid.

The course of the fluorescence spectra excited by a He-Ne LASER is at the room temperature similar to the spectra excited by conventional light source. The Stokes' shift between the band maxima makes 11 nm. If we narrow the slit of the monochromator several peaks appear on the curve of fluorescence course (see fig. 2). The positions of the peaks are shown in Table 1.

The course of the fluorescence spectra measured at the liquid nitrogen temperature is quite different. The mirror symmetry disappears and the intensity of the longwave band increases as a function of the dye concentration (see fig. 3, 4). The values of ratios of the both bands intensities normalized to equal exciting intensity are shown in Table 2.

Recently some properties of polymer foils containing chlorophyll a were measured [13], [14]. Thdve aantage of the study of these systems is their certain similarity to the systems in which the chlorophyll molecules of the living tissues are built.

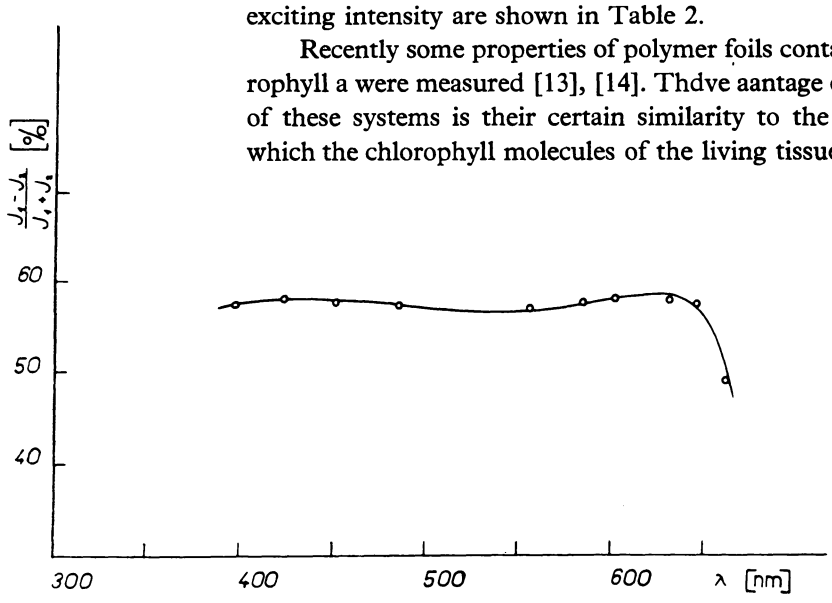

Fig. 1. The ratio of the difference of both fluorescence bands intensities to their sum plotted against the excitation wavelength

Table 1.

Observed peak [nm]	645	654	671	672,5	674	698
Characteristic Raman scattering line [cm^{-1}]	301	513	901	928	957	1 472
Raman scattering of the laser line 632,8 nm [nm]	645,2	654,2	671,2	672,5	673,8	698,0

We prepared two kinds of polymer foils: polystyrene and acetylbutyrate*) with the chlorophyll concentration of about 10^{-5} M and measured their absorption and fluorescence spectra. The absorption spectrum of both foils is approximately the same with wavelengths of band maximums for polystyrene $\lambda_S = 436$ nm, $\lambda_R = 669$ nm and for acetylbutyrate $\lambda_S = 433$ nm, $\lambda_R = 667$ nm. The spectral shift to the red of 3–6 nm of the Soret band maximum and 5–7 nm of the red band maximum corresponds to the expected effect of the interaction between chlorophyll molecule and a chain of polymer molecules. While in solution the chlorophyll very easy decomposes it is relatively very stable in a polymer foil. The fluorescence spectrum of polystyrene foil excited with He-Ne LASER at the room temperature has again mirror symmetry with respect to the longwave absorption bands with maximum wavelengths of $\lambda_1 \sim 680$ nm, $\lambda_2 \sim 740$ nm. The ratio of both maxima intensities is $E_{\lambda 1} : E_{\lambda 2} = 2.70$ (layer thickness of 0.30 nm). The yield is much less with respect to the solutions due to the small layer thickness and also to the probable quenching effect of polymer molecules.

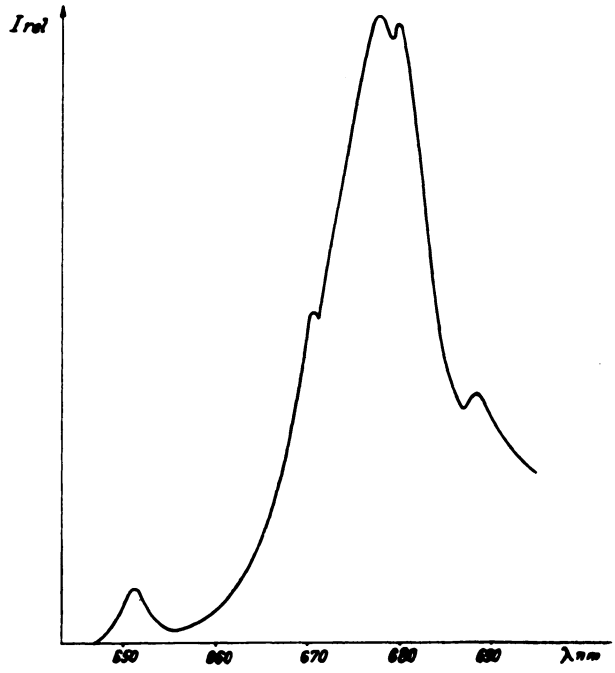

Fig. 2. The structure of the chlorophyll a emission band wit h $\lambda_{max} = 678$ nm. Ethanol-ether (1 : 1 vol) solution, $c = 1{,}04 \cdot 10^{-4}$ M, $t = 22$ °C

*) We recieved the pure primary material by courtesy of the Department of Organic Technology of the Chemical-Technological University of Pardubice.

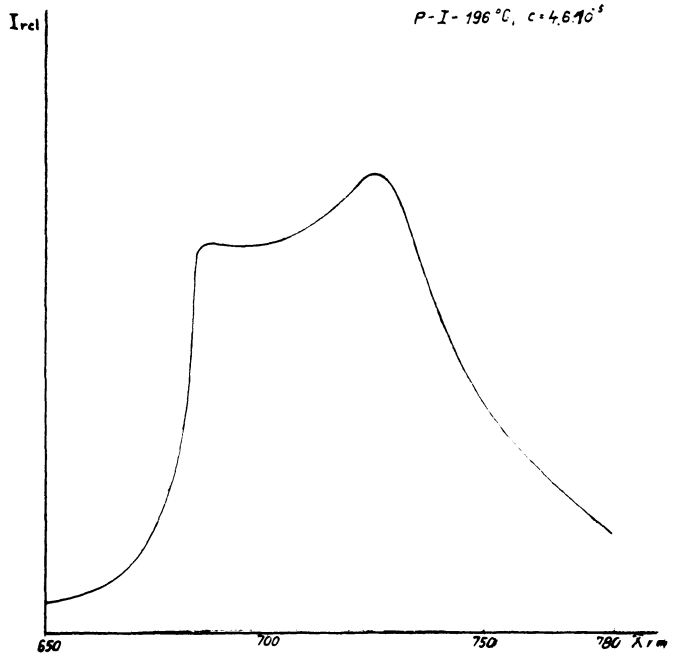

Fig. 3. The fluorescence spectrum of chlorophyll a solution in 3 – methyl pentane; c = 4,6 . 10⁻⁵ M, t = —196°C

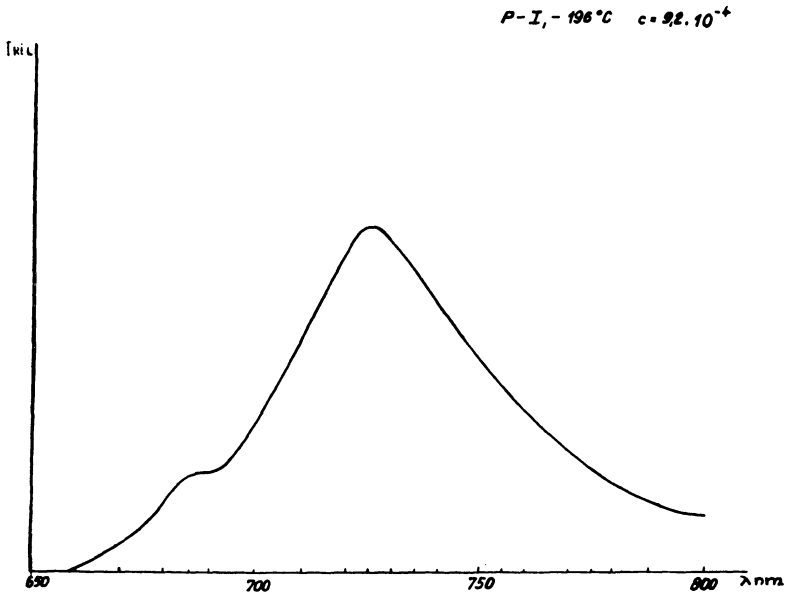

Fig. 4. The fluorescence spectrum of chlorophyll a solution in 3 – methyl pentane; c = 9,2 . 10⁻⁴ M, t = —196°C

Table 2.

	22 °C	−196 °C	
Chlorophyll concentration [M]	$4.6 \cdot 10^{-5}$	$4.6 \cdot 10^{-5}$	$9.2 \cdot 10^{-4}$
$I_{673} : I_{720}$	2.0	0.86	0.28

3. Discussion

From the above mentioned results the structure of the fluorescence band observed by the LASER excitation allows the easiest interpretation. All peaks are well separated from the exciting LASER line and lie on the longwave side of it. Therefore there was not any maximum observed that could be attributed to a radiant transition from a nonzero vibronic level of the lowest excited singulet state. We compared the wavelengths of observed peaks with the wavelengths of Raman scattering LASER lines in solvent used (3-methyl pentane) and found good agreement. Therefore we concluded that the observed structure did not belong to the chlorophyll but it results from the supersposition of the fluorescence spectrum of chlorophyll and the Raman scattering lines of the LASER line in the solvent.

The agreement between the absorption and excitation spectrum indicates that the energy levels taking part in the absorption of exciting energy cannot belong to more than one species of chlorophyll molecule. As we do not observe in the absorption spectrum any band (not even a smallest shoulder) that could be attributed to dimers or higher oligomers this species must be only the chlorophyll monomer. But the question of emitting species remains until now open. The assumption of the longwave fluorescence band being the second vibronic band of the basic electronic transition is in contradiction with the low temperature fluorescence spectra measurements done by Broyde and Brody [1], [2] and with our measurement too. The behaviour of the longwave fluorescence maximum at the low temperature and higher chlorophyll concentration suggests that there exists another chlorophyll species with competitive effect to the chlorophyll monomer absorbing the excitation energy and emitting near 670 nm (with probable vibronic satellite near 700 nm). Broyde and Brody [1] supposed the cooperation of monomer emitting in shortwave band and dimer emitting in longwave band and so they indirectly supposed the energy transfer from monomer to dimer. However they did not present any direct experimental prove of presence of dimers in their samples. We did not succeed in any experimental prove of dimers in our samples too. Another possible explanation is to assume the interaction between two neighbouring chlorophyll molecules one of them is excited (photodimer). The spectral shift of 1050 cm^{-1} between both fluorescence bands can be attributed to this interaction. This process can appear in fluorescence spectrum only and cannot appear in absorption spectrum. This conclusion is supported by the measurements of fluorescence polarisation spectrum of chlorophyll

a done by Kravcov [15] and Kaplanová [16]. In the time being the question which of the proposed mechanismus in fact takes place cannot be solved. Further experimental studies are necessary.

From the measurements made on foils only preliminary qualitative conclusion can be drawn that any substantial difference of the absorbing dye molecule with respect to the solutions does not appear. The only varying quantity is the interaction of the dye molecule with its surroundings which lowers the chlorophyll energy levels with respect to its state in solution. We can suggest that the acting species is in this case again a monomer and the mechanism of the emission should be probably similar to that in solution. The interesting phenomenon pointed out by Seely [13] is lowering the fluorescence yield in polymer foils. This is the subject of further studies.

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