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Difference Absorption Spectrum of Chlorophyll *a* in Polar and Non-Polar Solvents

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The absorption curves of chlorophyll *a* were measured in non-polar and polar solvents by the method of difference spectra for various dye concentrations in the range of 10^{-3} — 10^{-7} M. The width of absorption bands in nonpolar solvent increases nonsymmetrically on the longwave side with increasing dye concentration. The direct observation of this effect in absorption spectrum is very difficult but it can be seen in difference spectrum very clearly. This width increase disappears after addition of very small amount of polar solvent. It was not observed in polar solvent. This effect can be explained by the formation of chlorophyll dimers which are violated in polar solvents due to the solvation.

Измерены спектры поглощения хлорофилла *a* в неполярных и полярных растворителях методом дифференциальных спектров при разных концентрациях пигмента (с 10^{-3} до 10^{-7} M). В неполярном растворителе полосы поглощения на длинноволновой стороне несимметрически расширяются в зависимости от концентрации. Это расширение очень плохо наблюдается в спектре поглощения, но в дифференциальном спектре оно явно выражено. После добавки маленького количества примеси полярного растворителя оно исчезнет. В полярном растворителе оно вообще не наблюдается. Этот эффект может быть объяснен появлением димеров хлорофилла, которые нарушаются в полярных растворителях под влиянием сольватации.

Byla provedena měření absorpce chlorofylu *a* v nepolárních a polárních rozpustidlech metodou diferenčních spekter pro různé koncentrace barviva v oboru 10^{-3} — 10^{-7} M. V nepolárním rozpustidle se objevuje s rostoucí koncentrací nesymetrické rozšíření absorpčních pásů na dlouhovlnné straně, které se velmi obtížně pozoruje v absorpčním spektru, ale v diferenčním spektru je jasně patrné. Toto rozšíření mizí po přidání velmi malého množství polárního rozpustidla. V polárním rozpustidle se nepozoruje. Tento efekt se dá vysvětlit vznikem dimerů chlorofylu, které se v polárních rozpustidlech narušují vlivem solvatace.

The absorption spectrum of chlorophyll *a* is well known and has been studied in many solvents (see e.g. [1], [2], [3], [4]). The positions of absorption-band peaks (Soret as well as red band) is affected by the solvent polarity. The shift of these maxima has been measured in solvents of various polarity and Seely and Jensen [5] tried to interpret this shift by the interaction between dye and solvent molecules.

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It was assumed generally that it was with increasing dye concentration only that the band halfwidth increased and the band peak position remained fixed. Amster and Porter [6] measured the absorption spectra of chlorophyll *a* dissolved in very pure and dry 3-methyl pentane and found besides the red absorption band a new absorption band shifted about 12 nm to the red. This band appeared as a small shoulder on the red side of the 666 band in less pure solvents and its height increased with the dye concentration increasing. They attributed this band to an aggregated form of chlorophyll *a* — a dimer. This band has not been observed in different conditions although the absorption spectra are measured steadily as main information on the purity of samples prepared.

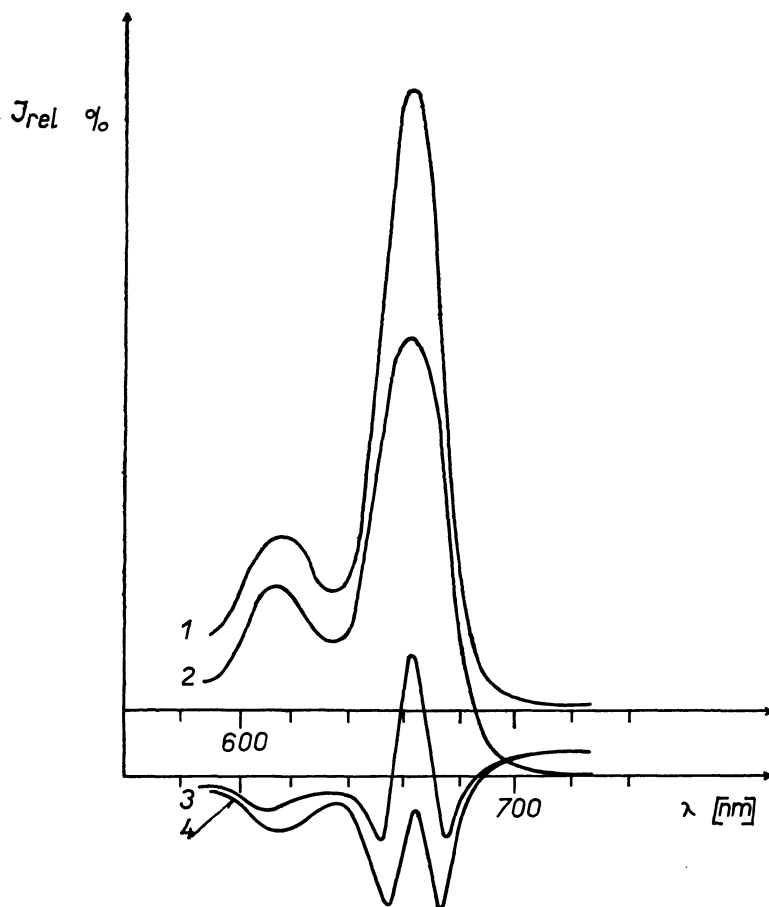


Fig. 1. Absorption and difference absorption spectrum of Chlorophyll *a* solution in acetone
 Curve 1 — absorption spectrum of the concentrated solution $c = 3 \cdot 10^{-5}$ M
 Curve 2 — absorption spectrum of the diluted solution $c = 6 \cdot 10^{-7}$ M
 Curve 3, 4 — difference absorption spectra

We studied the absorption spectra of chlorophyll solutions in polar as well as non-polar solvents and in various dye concentrations. The assumption about the fixed positions of the band peak positions was proved for polar solutions in wide range of chlorophyll concentration (10^{-3} — 10^{-7} M). The width of the band increased symmetrically with respect to the peak position. But for nonpolar solvents — such as 3-methyl pentane — the band increases its width asymmetrically with the concentration increasing. The peak shift lies at the limit of the apparatus sensitivity (see Fig. 1). Therefore we measured the difference absorption spectra (DAS) on the double — beam CF-4 Optica Milano spectrophotometer in the two samples that differ in chlorophyll concentration in the ratio 1 : 50 (the basic solution: $c =$

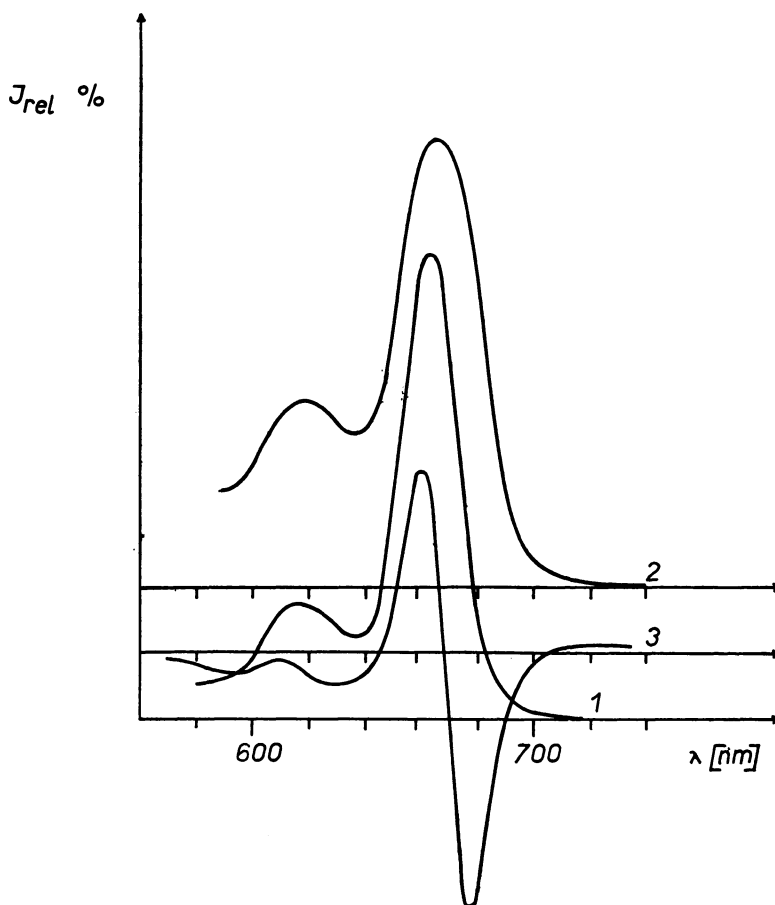


Fig. 2. Absorption and difference absorption spectrum of chlorophyll a solution in 3-methyl pentane
 Curve 1 — absorption spectrum of the concentrated solution $c = 2 \cdot 10^{-5}$ M
 Curve 2 — absorption spectrum of the diluted solution $c = 4 \cdot 10^{-7}$ M
 Curve 3 — difference absorption spectrum

$\approx 2 \cdot 10^{-5}$ M, the diluted solution: $c = 4 \cdot 10^{-7}$ M). The DAS of chlorophyll *a* in 3-methyl pentane on the Fig. 2. clearly shows the increased absorption of the diluted solution on the shortwave side of the red absorption band and the increased absorption of the concentrated solution on the longwave side of this band. When the concentration of one solution decreases the corresponding part of the absorption band diminishes. If we add a small amount of polar solvent (one drop i.e. 0.1% of acetone or ethyl alcohol) to the concentrated solution, the longwave extension of the absorption band disappears instantaneously. Similar effects can be observed in the Soret band but they are more complicated due to the structure of this band. We can compare these effects in 3-methyl pentane with the DAS of chlorophyll *a* in acetone measured with similar conditions (the basic solution $c = 3 \cdot 10^{-5}$ M). The symmetry of the band does not change and no effect of small impurities is observable.

These effects cannot be accounted for by the interaction between the dye and the solvent molecules only. This interaction plays undoubtedly an important role as can be seen from the strong effect of the slight impurities of the polar solvent. We can assume that this interaction predominates in solutions of polar solvents and that in these solutions all dye molecules are solvated. In non-polar solvents this interaction is obviously weak and in more concentrated solutions the interaction between the dye molecules themselves prevails and dimers or higher aggregates arise. This interaction must appear also in the solvents of commercial R.P. purity but its effect cannot be observed due to the solvation effect of polar impurities when they are present in concentrations comparable to the dye concentration. The method of the difference absorption spectra provides the way how to increase sensitivity of the apparatus for measuring of these effects.

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