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

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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 bands 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 interaction between dye and solvent molecules. It was supposed generally that with increasing dye concentration only 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 beside 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 increasing dye concentration. They attributed this band to a aggregated form of chorophyll *a* — a dimer. This band has not been observed in different conditions although the absorption spectra are measured steadily as a main information about the purity of prepared samples.

We studied the absorption spectra of chlorophyll solutions in polar as well as non-polar solvents and various dye concentrations. The solvents were of commercial p.a. purity as well as spectroscopic purity i.e. especially the non polar solvents were disposed of polar impurities. The assumption about the fixed positions of the band peaks 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 — as e.g. 3-methyl pentane — the band increases its width asymetrically with increasing concentration. The peak shift lies in 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 on the two samples that differ in chlorophyll concentration in the ratio 1 : 50 (the basic solution: $c = 2.10^{-5}$M, the diluted solution: $c = 4.10^{-7}$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

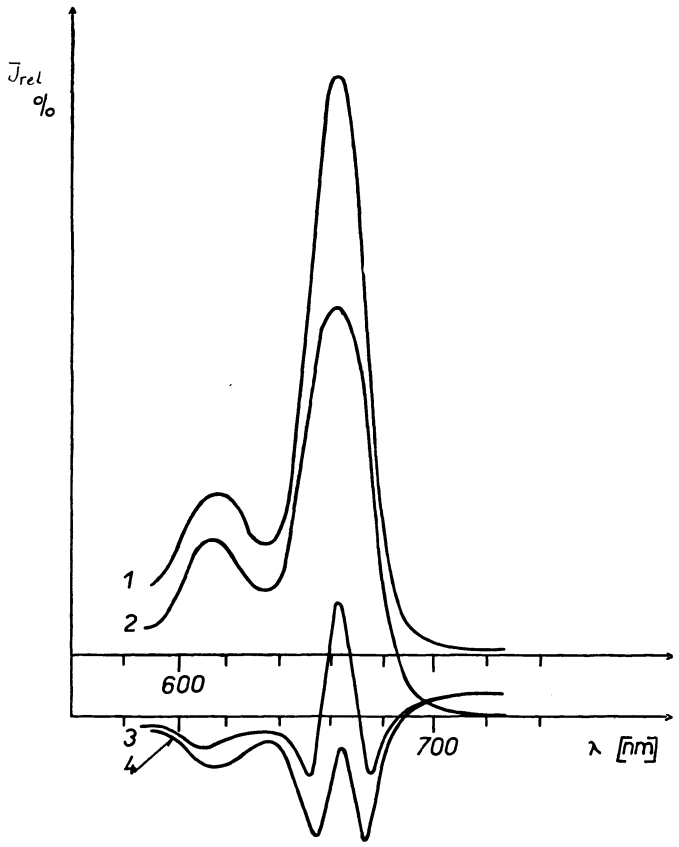

Fig. 1. Absorption and difference absorption spectrum of chlorophyll *a* solution in acetone Curve 1 — absorption spectrum of the concentrated solution $c = 3.10^{-5}$ M Curve 2 — absorption spectrum of the diluted solution $c = 6.10^{-7}$ M Curve 3, 4 — difference absorption spectra

can be observed on 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 $c = 3.10^{-5}$M). The symmetry of the band does not change and no effect of small impurities is observable. Similar but much weaker effects can be observed in solvents of p.a. purity.

These effects cannot be interpreted by the interaction between the dye and solvent molecules only. This interaction plays undoubtedly an important role as can be seen from

the stark effect of the slight impurities of 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

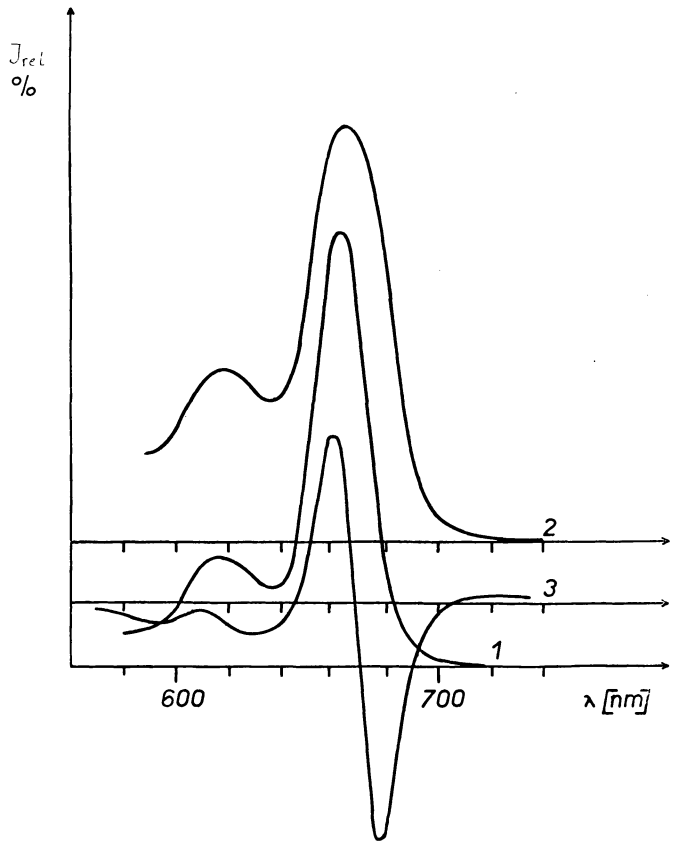

Fig. 2. Absorption and difference absorption spectrum of chlorophyll *a* solution in 3-methyl pentane Curve 1 — absorption spectrum of the concentrated solution $c = 2.10^{-5}$ M Curve 2 — absorption spectrum of the diluted solution $c = 4.10^{-7}$ M Curve 3 — difference absorption spectrum

dimers or higher aggregates arise. Therefore these effects can be attributed to solvate — dimer equilibrium in polar and non-polar solvents. This method provide us the sensitive tool for the observation of small dimer concentrations that are out of sensitivity range of absorption spectra measurements and therefore the presence of dimers can be traced on medium sensitivity spectral apparatus.

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