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The Electrodeposition of Films of Chlorophyll-a Microcrystals and Their Spectroscopic Properties†

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It is found that a suspension of microcrystals of chlorophyll-a (Chl-a) in a nonpolar medium may be electrodeposited as a film on a cathode. Spectroscopic properties of the film indicate that it is quite uniform and consists of randomly oriented microcrystals. Upon heating, the electrodeposited microcrystalline film is transformed to an alternate form of aggregated Chl-a. This new film appears to resemble very closely the Chl-a films previously obtained in monolayer work. Either film may be dissolved into monomer form and it is seen how Chl-a monomer is conserved during the complete process proceeding from electrodeposition to thermal conversion and the final dissolution. Furthermore, it is found how the oscillator strength over the red-visible region is the same for the dissolved monomer and the second type of film. The simple excited state resonance model for the spectrum of the latter is thus supported. The oscillator strength of the microcrystal film, by contrast, is about 40% greater. Such electrodeposited films are quite stable at room temperature and can serve well as samples in a variety of photoelectric studies.

INTRODUCTION

It has been known that chlorophyll-a (Chl-a) in nonpolar hydrocarbon solvents exists in monomeric as well as in aggregated forms. Earlier work by Jacob, Vather and Holt[1] has established the importance of water in preparing the microcrystalline form of Chl-a, which is distinctly characterized by its strongly red-shifted absorption band at 740-745 nm. Later studies on solid chlorophyll-a in dried films or in monolayer forms by Shermin and Linschitz[2], Litvin and

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Gulyaev[3] and Krasnovskii and Bystrova[4] indicate that water has a strong effect on their absorption spectra. Depending on the history of the chlorophyll-a samples and the equilibration time with water vapor, the red absorption peak of the solid chlorophyll-a can be shifted between 673 nm and 745 nm, apparently corresponding to various states of aggregation of Chl-a and Chl-a / H_2O. More recently, extensive studies by Katz and coworkers[5] lead them to conclude that the 745 nm microcrystalline form of Chl-a is actually a one to one Chl-a$\cdot H_2O$ adduct. These microcrystals also exhibit some interesting photo electric[6,7] and electron spin resonance[8] properties which are not observed in monomeric Chl-a.

In the course of investigating the photoelectric properties of this microcrystalline form of Chl-a we came to discover that in hydrocarbon liquid such as 3-methylpentane (3-MP), it is capable of being electro-deposited as a positively charged particle on an electrode surface. A fairly uniform film is formed. Thus a physical separation of the microcrystalline form from the monomer (or small aggregate) in a true solution is accomplished. The purpose of this note is to report the technique of electrodeposition and some spectroscopic properties of this form of Chl-a film. In what follows, the terms microcrystalline form, or simply microcrystals, shall refer to the ordered structure of Chl-a having an absorption peak at 740-5 nm and in which water seems to be an important component.

EXPERIMENTAL

Chl-a was extracted from fresh spinach by the method of Strain and Svec.[9] Crystallization was done by the method of Jacob *et al.*[1] The purity of this form of crystalline obtained was checked by thin layer chromatography. The visible absorption spectrum in ether agreed well with the literature. Samples of Chl-a obtained from Sigma Chemical Co. were also used and were found to have similar properties. The 3-MP was 99+% mole pure grade from Phillips Petroleum Co. It was purified by storing over molecular sieves (equal portions of 5x, 10x, 13x, sieves from Linde). A typical suspension/solution of Chl-a in 3-MP was prepared by simply sonicating a 10 mg sample of Chl-a in 50 cc of 3-MP for 2-5 minutes. The large particles, not in suspension, were removed by centrifugation.

Electrodeposition was done in a sandwich cell originally designed for photoelectric studies. The cell consists of two parallel electrodes where the front electrode is made of a semitransparant chromium coated quartz disc (2" diameter). The back electrode is of polished aluminum. The spacing between the two is varied between .005 to .250 inches, using mylar or teflon rings with an inner diameter of 3/4 inch. A suspension/solution of Chl-a in 3-MP was introduced within the spacing ring. To electrodeposit the microcrystals of the Chl-a on the quartz electrode, a high positive D.C. voltage giving a field strength on the order of 1 kv per cm within the cell was applied to the aluminum electrode (anode) for 2-5 minutes. The positively charged microcrystals migrated towards the quartz electrode (cathode) forming a fine film of microcrystalline Chl-a on the surface. To increase the surface concentration or thickness of the film, either a thicker spacer or a more concentrated solution was used. Films of surface concentration between 0.5 to 20 x 10^{15} molecules per cm^2 can thus easily be obtained. Surface concentration was determined by dissolving the film in 5 cc of diethyl ether and by measuring the optical density of the resulting monomeric solution, where the extinction coefficient is well known.

Electrodeposition was also carried out in an ordinary 10 mm path cuvette. Two rectangular strips of Cr coated semitransparent quartz were used as electrodes. In this arrangement the migration of the Chl-a microcrystals can be directly monitored in a spectrophotometer.

The suspension/solution can always be converted to the pure solution by simply heating to 50°C or by an addition of a drop of polar solvent such as methanol. The microcrystals then dissolve (into monomer or small aggregates) and efforts at electrodeposition from such solution proved entirely negative.

All spectra reported here were obtained with a Cary 14 spectrophotometer.

RESULTS AND DISCUSSIONS

A suspension/solution of Chl-a in 3-MP is fairly stable at room temperature but decomposes readily into monomeric and small aggregate forms at temperature

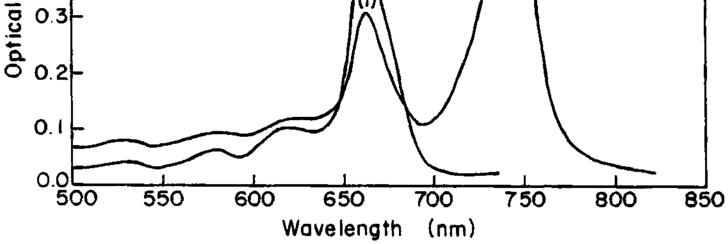

FIGURE 1. Absorption spectra of a suspension/solution of chlorophyll-a in 3-methylpentane: (1) at room temperature, (2) at room temperature after having been heated to 50°C for a few minutes.

above 35°C. Typical absorption spectra of such a suspension/solution are shown in Figure 1. The intensity of the absorption peak of the microcrystals at 745 nm decreases to zero with increasing temperature and simultaneously there is a growth of intensity of the monomeric peak at 662 nm. A spectrum of a pure suspension (with no monomers) in 3-MP has not been seen by us, for monomeric (small aggregate) Chl-a invariably coexists with the microcrystalline form in the suspension/solution. However, as might be expected, the electrodeposited film of the microcrystalline form of Chl-a on the quartz electrode exhibits an almost pure microcrystalline type of spectrum. It has a far red absorption band at 740-2 nm (a slight blue shift) and a Soret band at 445 nm. The spectrum resembles that reported by Jacobs *et al*, for a suspension of the microcrystalline form of Chl-a in mineral oil.[1] On heating the electrodeposited film, a band at 675 nm grows in strength at the expense of the intensity of the microcrystal band at 740 nm indicating the breakdown of microcrystals. Unlike the suspension in 3-MP solvent, the film is thermally more stable; complete breakdown of microcrystals occurs only at temperatures above 70°C. Absorption spectra of a typical electrodeposited film are shown in Figure 2. The spectral data of either film – the 740 nm form and the 675 nm form – are summarized in Table I.

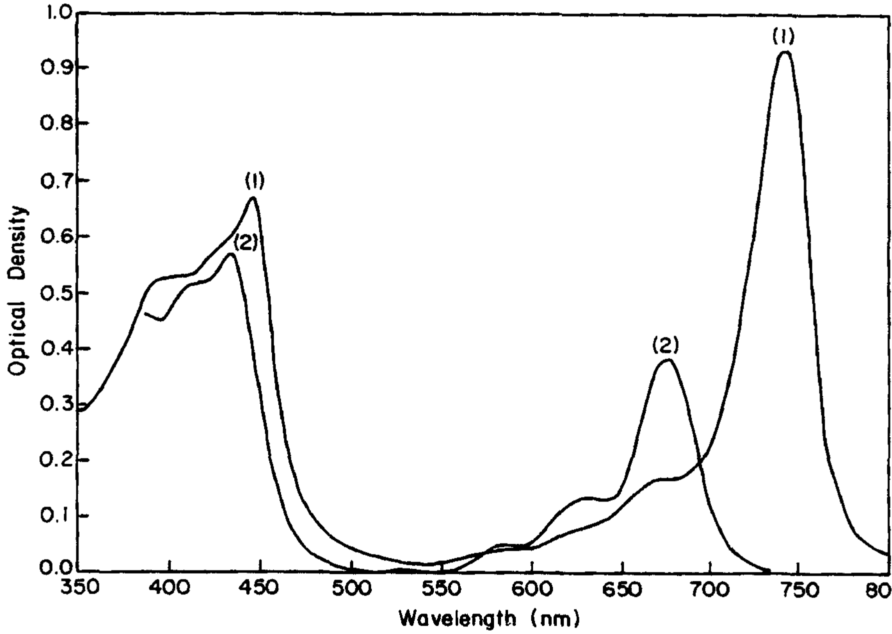

FIGURE 2 Absorption spectra of a chlorophyll-*a* film electrodeposited on a semitransparant chromium coated quartz electrode: (1) at room temperature, (2) at room temperature after having been heated to 70°C for a few minutes.

TABLE I

Spectra data of an electrodeposited chlorophyll-a film

State of chlorophyll-a film	Soret band band position	Red band band position	Half width	Ratio of O.D. Soret/Red
740 nm form	447 nm	740–743 nm	38–40 nm	.70– .75
675 nm form	435 nm	675–678 nm	43–45 nm	1.42–1.50
Monolayer[a]	435 nm	675 nm	~45 nm	1.38

[a] from reference 11

X-ray diffraction patterns have also been obtained from the electrodeposited Chl-a film (Figure 3). The 740 nm form shows a well defined diffraction pattern demonstrating its ordered nature. In contrast, the 675 nm film does not exhibit any diffraction pattern at all. Evidently, the thermal breakdown of the microcrystals leads to a disordered structure. The powder X-ray diffraction pattern of Chl-a crystals has been previously reported by Anderson and Calvin.[10] It has the same structure as that reported here for the microcrystalline film with only some apparent differrences in the relative intensities.

The electrodeposited film of the microcrystalline form of Chl-a (740 nm form) is rather insoluble in 3-MP, but is readily soluble after it has been converted to the alternate (675 nm) form at 70°C. However, either film, the 740 nm form or the 675 nm form, is readily dissolved with diethyl ether yielding a solution with a typical monomeric Chl-a absorption spectrum.

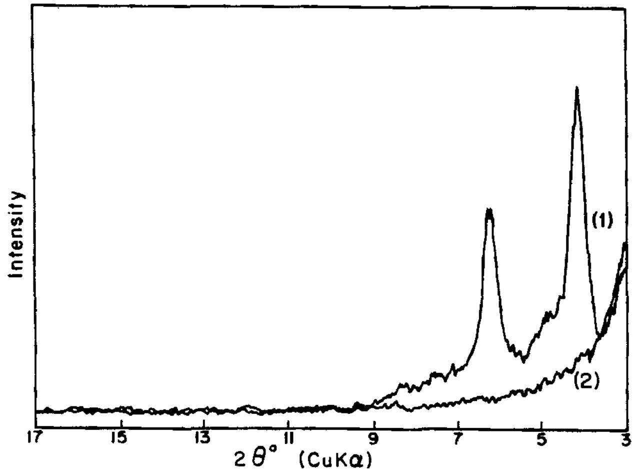

FIGURE 3. X-ray diffraction pattern of chlorophyll-a film electrodeposited on a semi-transparent chromium coated quartz electrode: (1) the 740 nm form, (2) the 675 nm form obtained after heating (1) to 70°C.

The optical density of the electrodeposited Chl-a film in either the 740 nm or 675 nm form is found to be proportional to its surface concentration. Linear plots of optical densities at 740 nm and 675 nm versus the surface concentration of the film are shown in Figure 4. The apparent "Beers Law" behavior of the Chl-a film supports the impression that it is homogeneous and optically thin. There follows the implication that the microcrystals themselves are optically thin as well.

There are studies in the literature of monolayer films of Chl-a prepared using a Langmuir trough[11]. The spectrum of such a film is very similar to that seen here for the 675 nm form of the electrodeposited Chl-a (see Table I). If the 675 nm film in the present studies represents an aggregated Chl-a, which however is microscopically *disordered*, then we might expect a conservation of oscillator strength upon going from pure monomer to this aggregate form. This would assume the simple excitonic, or excited state resonance theory, for the system in which aggregation simply spreads out the monomer oscillator strength over a spectral region whose width depends on the geometric arrangement of the monomers in the aggregate. In order to test these ideas, we have determined visible absorption band areas for the 675 nm film and for the monomer solution

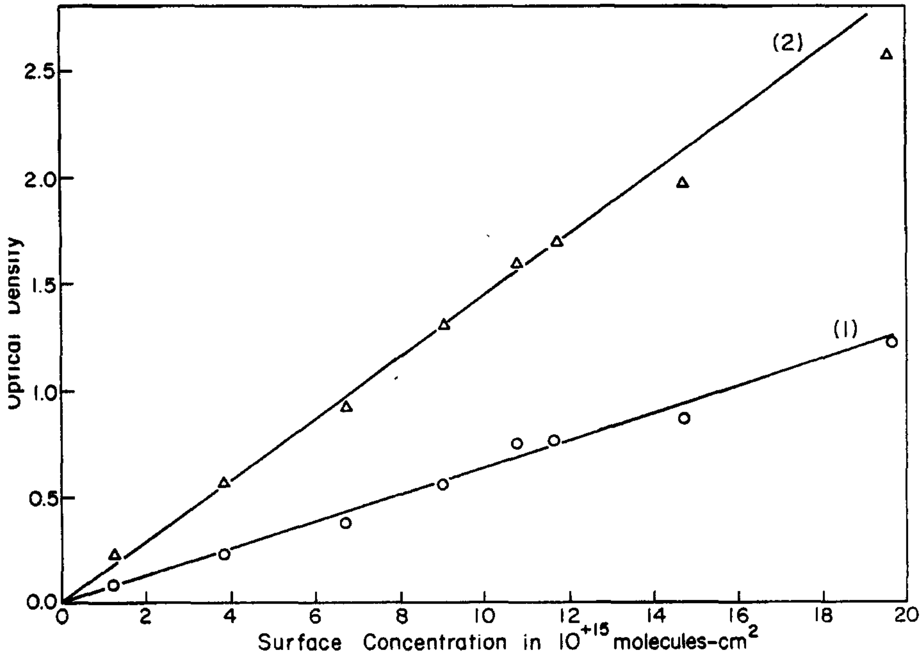

FIGURE 4 A optical aptical density versus surface concentration of a chlorophyll-a film. (1) OD (675 nm) (2) OD (740 nm).

made by dissolving this film. The integration is on a frequency scale and is extended from 800 nm - 500 nm to include all vibrational structure and, in the case of the aggregate form, to include a possible distribution into the several resonance-split states. Such analyses are performed for several surface concentrations and the results appear in Table II. A conservation of oscillator strengths is apparent to within 10%. This is an important observation. Not only does it, incidentally, confirm optically thin and homogeneous properties of the film, it also suggests that the standard resonance picture is valid for the aggregate and that these aggregates are *randomly* arranged in the film just as surely as are the monomers randomly arranged in solution. Thus, the red shifted but weakened peak at 675 nm of the aggregate must have a blue shifted component as well. More detailed resolution of the spectrum would lead to information about the molecular arrangement within the aggregate form.

In contrast to the oscillator strength conserving 675 nm aggregate, the spectral behavior of the microcrystalline form is different. First, however, we note two important points. When a suspension of the microcrystals is viewed with the light beam of the spectrophotometer parallel to the applied field, no significant change in the optical density of the 740-5 nm band is seen during the act of electrodeposition. (However, when it is viewed transverse to the field, this far-red band is seen to disappear as the microcrystals now move out of the light path of the spectrophotometer). Assuming that the microcrystals themselves are dichroic, this can only mean that they are deposited in a disordered fashion; for almost any type of uniform ordering would have to alter the optical density. This disorder, then, agrees with the disorder just argued for the 675 form of the film. (Strictly speaking, that earlier argument, too, is viable only under the assumption that a single aggregate species is optically anisotropic). However, the disorder in the 675 nm form might have been induced by the heating needed to

TABLE II

Oscillator strength of the 660 nm band of chlorophyll-a in ether solution and of the 675 band of chlorophyll-a film calculated for a set of Chl-a samples

Sample No	Surface concentration of Chl-a film Molecules/cm^2	Oscillator[a] strength of the 660 nm band of Chl-a solution	Oscillator[a] strength of the 675 nm band of Chl-a film
1	1.24×10^{15}	.31	.34
2	3.80	.29	.29
3	6.74	.24	.24
4	9.05	.22	.24
5	10.78	.27	.28
6	11.67	.25	.26

[a] By integrating over the broad range of visible absorption. The usual formula, $f = 4.3 \times 10^{-9} \int \epsilon d\nu$, has been used *without* any correction for index of refraction.

convert the microcrystalline film. Secondly, we note that the act of microcrystal electrodeposition followed by thermal conversion to the 675 nm aggregate and subsequent dissolution to monomer, conserves monomeric Chl-a (i.e. no Chl-a destruction). Since electrodeposition is not accompanied by any significant change in the optical density of the 740-5 nm band, no monomeric Chl-a is lost in that step. Furthermore, when identically electrodeposited films are dissolved to monomer before and after heating (conversion to 675 nm form) the monomer optical density is also conserved. Thus, we can infer that the full cycle of electrodeposition, thermal conversion, and dissolution does not destroy any significant amount of Chl-a.

When areas are integrated for the 745 nm microcrystal band we find that the oscillator strength (per monomer) carried by this band is about 40% greater than carried by the monomer (or the 675 nm aggregate) band. Apparently the resonance picture is now simply not valid and an essentially new electronic transition is indicated. This observation would seem to agree with the recent insight into the structure of the Chl-a microcrystals where the $(\text{Chl-a} \cdot H_2O)_n$ type adduct is indicated.[5] On the other hand the species absorbing a 675 nm is the oligomer $(\text{Chl-a} \cdot \text{Chl-a})_n$ the dehydrated and disordered form of the Chl-a-water adduct. If it turns out that the H_2O is bound in such a way as to seriously perturb the chromophore, perhaps in an acid-base sense, then such basic electronic changes are not unexpected. The thermal conversion of the microcrystalline film on the electrode to the 675 nm film then corresponds to its dehydration.

It is of course, interesting how the majority of microcrystals in suspension in the low dielectric constant medium, 3-MP, all carry a net positive charge. The cathodic electrodeposition is more than 95% complete. In fact, when the transparent electrode is made the *anode*, no detectable Chl-a optical density appears. It is known[12] that a colloidal suspension of particles such as carbon black, or sulfur in hydrocarbon liquid, with added dispersant or a peptizing substance, is quite stable. The colloidal particles are either all negatively or all positively charged. In general the total net charge density in such systems cannot exceed that of "static electricity" in insulating environments. The Chl-a microcrystals at some point in their history have evidently acquired such a static charge which happens to be positive in sign. A referee of this paper has suggested that the possible charged species may be $(\text{Chl} \cdot H_2O)_n \cdot {}^{+}O_2$. We have repeated the electrodeposition experiments with Chl-a suspension/solution purged in one case with prepurified dried Nitrogen and in another case with Oxygen. We found that in both cases the electrodeposition is more than 95% complete and we do not observe spectrophotometrically any significant difference in the rate of microcrystal migration. These experiments probably indicate that physically adsorbed oxygen is not important in determining the charge on the microcrystal. Whether or not a more firmly bound oxygen plays an important role remains to be seen.

A more detailed study of the origin of the charge is beyond the present investigation.

There is mention in the literature of the phenomenon of photoeletrophoresis as applied to direct color-imaging systems.[13] It appears that the basic process there – the motion of charged pigment particles in an insulting medium in an electric field – is fully analogous to what is being observed for the Chl-a microcrystals.

CONCLUSION

In conclusion, the electrodeposition technique appears to provide a useful method for preparing a homogeneous film of randomly oriented microcrystals of Chl-a. Furthermore, heat can convert this film to a form (the 675 nm form) similar to that previously studied as Chl-a monolayers or anhydrous Chl-a films. It is found how the total oscillator strength of the 675 nm species in the 550 nm - 800 nm region matches that of the Chl-a monomer in the 500 nm - 700 nm. A resonance or excitonic picture of the excited states of the aggregate is consistent with this. The 745 nm electronic transition of the microcrystal has a significantly stronger oscillator strength which may relate to the proposed presence in the microcrystal of one water molecule to one Chl-a molecule.

From the point of view of photoelectric studies, the electrodeposited microcrystalline film offers an especially convenient and stable sample. The coated cathode can be dried and used reproducibly in a photoelectric cell in which the medium can vary from vacuum to gas, or from aqueous solutions to solutions in nonpolar solvents. In a 3-MP environment the microcrystalline film exhibits the same dual "fast" and "slow" photoelectric signals previously reported[7]. It appears that the 675 nm form of the film on the electrode also provides a good sample for such photoelectric studies. Preliminary results reveal signals (when moving the excitation to the 675 nm region) which seem to be weaker than in the microcrystal case.

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