Specific in vivo absorption coefficient of chlorophyll \( a \) at 675 nm\(^1\)

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**Abstract**

Spectral absorption of phytoplankton from cultures and natural samples was measured by means of an integrating-sphere photometer. Measurements on suspension yielded specific absorption coefficients for healthy phytoplankton cultures in collimated light at 675 nm between 0.007 and 0.013 \( m^2 \text{(mg Chl } a \text{)}^{-1} \). These values were independent of cell density and cell wall material.

Natural samples of Kiel harbor water yielded specific absorption coefficients at 675 nm of 0.009 and 0.017 \( m^2 \text{(mg Chl } a \text{)}^{-1} \). The specific absorption coefficients in suspension were dependent on the optical properties of the individual cells (packaging effect), decreasing (ca. \( 1/r \)) with increasing cell size (\( r \text{ radius} \)) and increasing chlorophyll \( a \) content per cell as predicted by theory, but are significantly lower than theoretically expected. Comparison with published values shows that specific absorption coefficients of cell suspensions are often overestimated, presumably because of methodological problems.

In the ocean, absorption of daylight by particles is dominated by phytoplankton pigments. This fact leads to remote-sensing algorithms where upwelling radiance from the ocean surface is interpreted in terms of Chl \( a \)-equivalent phytoplankton standing stock (Gordon 1980; Platt and Herman 1983; Eppley et al. 1985). This approach assumes constant absorption coefficients of phytoplankton per amount of extractable Chl \( a \). For much the same reason Chl \( a \) has served as a reference parameter for potential primary production in biological oceanography (Eppley et al. 1985) and to indicate physiological status (e.g. assimilation number). Apart from the possible influence of variable ratios of accessory pigments to Chl \( a \), there is no evidence in the ecological literature that the concept of a constant specific absorption coefficient does not hold in situ. Another reason for the popularity of Chl \( a \) as a reference parameter is the ease of measurement by in vitro methods that are generally accepted and accessible to most laboratories (Jeffrey and Humphrey 1975).

The measurement of quantitative in vivo absorption spectra has been approached by various methods (cf. Maske and Haardt 1987). Published values for Chl \( a \)-normalized, in vivo absorption coefficients \( [m^2 \text{(mg Chl } a \text{)}^{-1}] \) of phytoplankton show considerable variability (Maske and Haardt 1987). Possible explanations could be methodological artifacts, pigment composition, or the absorption characteristic of the individual cells, their optical density, and diameter (packaging or “sieve” effect: Duysens 1956).

The traditional experimental approach to measuring the absorption of scattering particles is to place the sample inside the Ulbricht (integrating) sphere, thus collecting all scattered light in addition to transmitted light. So far no measurements of samples at natural concentrations inside an integrating sphere have been presented because the low pigment concentrations compared to water absorption \( a_w(675) = 0.418 \text{ m}^{-1} \), and in absolute values call for extremely high resolution of radiative quantities.

Methodological problems can be partly overcome by using highly concentrated samples and calculating absorption from transmittance and reflectance. These parameters can be measured by placing integrating spheres between sample and detector (Grum and Becherer 1979) and between light source and sample. Because light scattered at a right angle within the cuvette will be partially lost, the light path within the cuvette needs to be kept short. Therefore this type of measurement is restricted to

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very thin and hence highly concentrated samples (Kiefer and SooHoo 1982). The originally parallel beam becomes diffuse within the sample and consequently light conditions of an unknown state of diffuseness have to be accepted, making them difficult to compare to absorption coefficients under collimated light.

The considerable technical effort of the above methods is made necessary because attenuation (extinction) spectra of a turbid sample obtained with a typical photometer are deformed, flattened, and generally much higher than absorption. In a photometer, attenuation (approximating extinction) is measured with a collimated light beam received by a detector of narrow acceptance angle (radiance detector); therefore only a small portion of the scattered light will be received. Besides, strong interference of scattering with absorption bands has to be expected. Loss due to scattered light generally increases with shorter wavelength (Hulst 1957; Haardt et al. 1979; Diehl and Haardt 1980).

Our research effort was directed toward evaluating in situ fluorescence as a source of a remotely sensed signal. We tried to estimate the variability and absolute value of in vivo absorption of phytoplankton and other particulate material. In this context the Chl a absorption band at 675 nm is of specific interest because of its interference with fluorescence emission at the 680–685-nm peak wavelength. The variability of literature data and results of comparative measurements of absorption with other methods (Maske and Haardt 1987) led us to measure absorption with an integrating sphere. To our knowledge these results are the first absorption coefficients measured at close to natural concentrations. We compare the specific absorption coefficients at 675 nm of various samples and relate them to their optical efficiency factors based on cell geometry and concentration of cellular Chl a.

T. T. Bannister advised us on the measuring protocol of the integrating sphere. We also acknowledge the technical help of C. Schomann and S. Neuer.

Methods

The absorption of suspended particles was measured in a flow-through cuvette (acrylate) 200 mm long and 40 mm in diameter.
Fig. 2. Light fluxes within the integrating sphere.

(C, Fig. 1) within an integrating sphere of 450-mm diameter (US, Fig. 1). The sphere was coated with barium sulfate and polyvinyl alcohol (99/1, w/w: Grum and Luckey 1968). The cuvette was continuously supplied during measurement by a peristaltic pump (Pu) from a sample reservoir whose content was agitated by a magnetic stirrer (MS), thus providing homogeneity of the particulate material of the suspension. Monochromatic collimated light (2-nm bandwidth, wavelength reading resolving 0.2 nm with an accuracy within 0.2 nm) was provided by the following setup (see Fig. 1): The light source (LS) (100-W tungsten-filament bulb, forced ventilation) is supplied by a constant current and illuminates the entrance slit of the grating monochromator (Mo), (Schoeffel: f/3.6, focal length 250 mm, dispersion 3.3 nm mm⁻¹) with wavelength drive (WD) via the heat filter (HF) and the collimating condensor (L₁). Second-order light is cut out by the alternate colored-glass filter (BF). The light collimated by lens L₂ is deflected by the mirror (Mi) through the dielectric beam splitter (BS, 90% transmission) and the diaphragm (D, diam, 15 mm). The entering light beam meets the cuvette at about 5° incidence angle, therefore the specularly reflected light, which is reflected at both ends of the cuvette, is directed to the inner wall of the sphere. The same is true for the main part of the forward scattered light which is reflected on the end of the cuvette. For this light flux the diaphragm is off axis, thus, in contrast to a perpendicular incident beam, only a negligible amount of the reflected forward lobe of the scattered light is excluded from detection. The 5° angle was chosen rather than a perpendicularly incident beam because, although the latter would exclude some of the specularly reflected light fluxes, the former allows calculation of the errors. For the perpendicular beam this is not possible in the case of natural samples because the errors due to the loss of reflected light through the diaphragm would depend on the angle width of the forward scattered lobe, which in turn would be defined by the cell size and refractive index of the sample particles.

In Fig. 2, four light fluxes within the integrating sphere are presented: F₀, flux entering the sphere; F₁, flux specularly reflected on the front of the cuvette; F₂, transmitted flux; F₃, flux specularly reflected on the end of the cuvette. If subsequent reflections are neglected, the detected flux (Fₘ) can be approximated by the sum of the three fluxes (F₁,F₂,F₃):

\[
F_m = F_0R + F_0(1 - R)\tau(1 - R + R\tau)
\]

where \( R \) is reflectance on the acrylate-air interface and \( \tau \) is transmittance within the sample suspension, \( \tau = \exp(-al) \). After solving the above equation for \( \tau \) and substituting fluxes by currents of the photodiode (\( I_m/I_0 = F_m/F_0 \)) we obtain

\[
\tau = \left[ \frac{I_m}{I_0R(1 - R)} \right]^{1/2} \cdot \left[ \frac{1 - R}{1 - R + R\tau} \right]^{1/2} + \frac{(1 - R)^2\tau_0}{4R^2}
\]

\( I_0 \) must be calculated from results obtained from distilled water (\( I_w \)):

\[
I_0 = \frac{I_w}{R + (\tau_w - R\tau_0)(1 - R + R\tau_w)}
\]

where \( \tau_w \) is known from the absorption coefficients of pure water \( (a_w \text{ at } 675 \text{ nm}) \) and the cuvette length. The reflectance, \( R \), was determined by filling the cuvette with com-
In vivo Chl a absorption

Table 1. Absorption coefficients computed with (cor) and without (uncor) correction for light reflected on the cuvette windows for different chlorophyll concentrations of one culture sample.

<table>
<thead>
<tr>
<th>Chl a (mg m⁻³)</th>
<th>α cor (m⁻¹)</th>
<th>α uncor (m⁻¹)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>3.232</td>
<td>3.115</td>
<td>-3.8</td>
</tr>
<tr>
<td>150</td>
<td>1.692</td>
<td>1.653</td>
<td>-2.3</td>
</tr>
<tr>
<td>75</td>
<td>0.859</td>
<td>0.846</td>
<td>-1.6</td>
</tr>
</tbody>
</table>

The detector (D₃) of the integrating-sphere photometer (Fig. 1) receives only diffuse light due to the baffle (Ba). To facilitate presentation in the drawing, we show the position of the detector and baffle 90° to the left. The signal (I₃) was registered as a ratio to the signal (I₉) of an independent light source monitor (D₉). Photodiodes (Centronics: F = 10 mm²) served as detectors. The data were obtained with a multiple ramp, integrating A/D converter. No noise of the ratio was observed above the 0.0002-OD level.

For determining α, the cuvette was filled with 0.2-μm Nuclepore-filtered samples (differential pressure, 0.2 bar) to exclude the absorption of water and Gelbstoff by calculation. Gelbstoff spectra were obtained by subtracting precise distilled water absorption data (Haardt and Diehl unpubl. results). The spectra of dissolved yellow substance showed no signature characteristic of phytoplankton pigments, excluding the possibility of spilled-over phytoplankton pigment in the reference absorption. One indication of the good accuracy of this method is the result that distilled water absorption corresponded within 0.002 m⁻¹ of the extinction measurement at 675 nm.

Chlorophyll concentration was measured by the trichromatic method (Jeffrey and Humphrey 1975). Samples were extracted in 90% acetone with glass beads in a homogenizer for 5 min. For Nannochloris sp. only, we extracted chlorophyll with methanol and glass beads at 80°C in a homogenizer. We calculated Chl a without subtraction of Chl b and Chl c absorption overlaps (Marker et al. 1980). Cells were counted and their size visually determined by means of an inverted (Utermöhl) microscope (10-ml counting chamber).

Cultures were grown in natural seawater.

In vivo Chl a absorption

Fig. 3. Absorption spectra of Cricosphaera carterae. The original sample of 300 mg m⁻³ was diluted to 50 and 25%. Absorption of sample-specific filtrate was subtracted. Absorption coefficient for cuvette length of 0.2 m.

Completely absorbing India ink. R was 0.042 at 675 nm and varied between a minimum of 0.024 at about 570 nm and 0.044 near 700 nm.

We calculated the true absorption coefficient by this method for our example shown in Fig. 3. This example covers the upper range of concentrations of our samples. We found decreasing error with decreasing concentrations (Table 1). Because the correction yielded negative deviation of <3.8% in our samples, we neglected this correction in our data.
of 30% (Nannochloris sp., 15%) with nutrients added at a concentration of f/20 (Guillard and Ryther 1962). Culture illumination by “cool-white” fluorescent bulbs was $1.5 \times 10^{-4}$ mol quanta $m^{-2} s^{-1}$ for bright-light-adapted cultures or $5 \times 10^{-6}$ mol quanta $m^{-2} s^{-1}$ for low-light-adapted cultures. Cultures were diluted at least once a week. The culture strains were obtained from M. Elbrächter (BAH, List) and were originally isolated from the North Sea. The cell sizes were: Thalassiosira eccentrica, 36–50-μm diameter, 17–40 μm high; Scrippsiiella trochoidea, 16-μm diameter, 23 μm long; Cricosphaera carterae, 10–12-μm diameter. Nannochloris sp. was obtained from an aquaculture project at Kiel/Bükk. Species composition of samples from Kiel harbor are given elsewhere (Maske and Haardt 1987).

**Theoretical considerations**

The absorption coefficient in an absorbing medium is defined by

$$-\frac{dF}{F} \sim \frac{dx}{a_{sus}}$$

where $F$ is flux and $x$ is length. In an absorbing medium the absorption loss of a beam of light is proportional to the path-length increment, with integration leading to Beer’s law. The factor of proportionality is the absorption coefficient, which in the case of a suspension ($a_{sus}$) can be divided into the components $a_w$ and $a_d$ (the absorption coefficients of water and dissolved matter) and $a_{part}$ (the absorption coefficient due to particles of the suspension):

$$a_{sus} = (a_w + a_d) + a_{part}.$$ 

We can derive $a_{part}$ from theory (Hulst 1957; Kerker 1969) for monodisperse suspensions of spherical, absorbing particles:

$$a_{part} = NAQ_{abs}$$

where $N$ is the number of particles per volume, $A$ the geometrical cross section of one particle, and $Q_{abs}$ the efficiency factor resulting from Mie theory. Phytoplankton particles in this framework can be approximated by spheres with equivalent volumes. If the deviation from sphericity is too strong, the particles can be corrected by a factor ($F$) accounting for the larger geometrical cross section of a statistically oriented nonspherical particle (Kirk 1976; Haardt et al. 1979):

$$a_{part} = N\pi r^2 F Q_{abs}. \tag{2}$$

For further evaluation we will assume spheres ($F = 1$) with radius $r$, homogeneous with respect to pigment distribution and refractive index ($m = n - in'$). The refractive index is close to 1 with respect to water, which is the case for phytoplankton cells even at bands of strong absorption (Reuter 1980). Therefore as a first approximation, scattering can be ignored with respect to absorption and the approximation of Hulst (1957) is applicable:

$$Q_{abs} = 1 + (ra)^{-1}\exp(-2ra) + 0.5(ra)^{-2}\left[\exp(-2ra) - 1\right].$$

$Q_{abs}$ is 1 in case of very large $r$ and $4ar/3$ for very small radii. $a$ denotes the absorption coefficient (units of $m^{-1}$) of the material within the particle. This formula is identical to the one by Duyens (1956) which was derived by applying principles of geometrical optics.

Dividing $a_{part}$ by the total pigment concentration $C$ of the particles in the suspension yields the specific absorption coefficient due to particulate matter in suspension $a^*_{part}$:

$$a^*_{part} = \frac{a_{part}}{K} \tag{3}$$

and

$$C = N\%\pi r^3 k \tag{4}$$

where $C$ is the pigment concentration within particles (mg m$^{-3}$). It follows from Eq. 2–4 that

$$a^*_{part} = \frac{3}{4C r} Q_{abs}. \tag{5}$$

The specific absorption coefficient of the particulate matter decreases with increasing size for large particles ($Q_{abs} = 1$), resulting in the well-known packaging effect.

$$a^*_{part} \sim 1/r$$

for $Q_{abs} = 1$. In the case of very small particles

$$\lim_{r \to 0} Q_{abs} = 4ar/3 \tag{6}$$

because

$$a = C_a a^*. \tag{7}$$

$a^*$ is the specific absorption coefficient of
Fig. 4. The ordinate shows the ratio \( a^{\text{norm}} \), measured specific absorption of phytoplankton suspension \( (a^{\text{part}}) \) to the specific absorption of a homogeneous solution of Chl a in vivo \( (a^{\text{ch}}) \). The abscissa represents \( 2r a^{\text{ch}C_t} \) of the individual phytoplankton cells. \( a^{\text{ch}} \) is assumed to be 0.02 m\(^2\) (mg Chl a \(^{-1}\)). Data from Privoznik et al. 1978—\( \Delta \); from Morel and Bricaud 1981—\( \square \); our data (cf. Table 2)—\( \circ \). Letters designate species (C—Cricosphaera carterae; N—Nannochloris sp.; S—Scripsiella trochoidea; T—Thalassiosira eccentrica). The filled circle indicates a dark-adapted culture. The continuous line was calculated according to Eq. 10.

The material of the particle we obtain from Eq. 5 and 6

\[
\lim_{r \to 0} a^{\text{part}} = \frac{3}{4rC_t} \lim_{r \to 0} Q_{\text{abs}}. \tag{8}
\]

By substituting Eq. 6 and 7 into 8 we obtain

\[
\lim_{r \to 0} a^{\text{part}} = a^{*}. \tag{9}
\]

\( a^{* \text{part}} \) (the specific absorption coefficient of the suspension due to particle absorption) thus can never exceed the value of \( a^{*} \), and the dimensionless ratio

\[
a^{\text{norm}} = \frac{a^{* \text{part}}}{a^{*}} \tag{10}
\]

ranges between 0 and 1.

Below, only specific absorption coefficients of Chl a are discussed where \( a^{*} \) at \( \lambda_0 = 675 \) nm was tentatively assumed to be

\[
a^{*} = a^{* \text{Chl}(675)} = 0.02 \text{ m}^{-1} (\text{mg Chl } a \text{ m}^{-3})^{-1}.
\]

In Fig. 4 \( a^{\text{norm}} \) vs. \( \rho' \), the product of \( 2r a^{* \text{Chl}C_t} \) (\( r = \) cell radius) is depicted. The function was calculated by substituting Eq. 5 into 10.

The above approach was derived by assuming homogeneous pigment distribution within spherical cells of volume \( V_0 \) and radius \( r \). Pigments in phytoplankton cells are actually concentrated in a number \( (z) \) of chloroplasts with diameter \( 2r_1 \) and volume of

\[
V_1 = \frac{V_0}{z} (1 - R_0)
\]

where \( R_0 \) is the ratio \( (V_0 - V_1)/V_0 \). One chloroplast has the radius

\[
r_1 = \frac{r(1 - R_0)^{\frac{1}{3}}}{z^{\frac{1}{3}}},
\]

and \( a_1 \), the absorption coefficient within the pigmented chloroplast, is calculated to be

\[
a_1 = \frac{C_1a^{*}}{1 - R_0}.
\]
Table 2. Culture parameter and in vivo absorption at 675 nm.

<table>
<thead>
<tr>
<th>Source</th>
<th>Diam $t$ (10$^{-6}$ m)</th>
<th>Chl a (mg m$^{-3}$)</th>
<th>Chl a (mg cell$^{-1}$)</th>
<th>$a'_{sw}$ $t$ ($m^2(mg\text{ Chl a})^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morel and Bricaud 1981</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platymonas suecica</td>
<td>6</td>
<td>$5.4 \times 10^{-10}$</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>Coccolithus huxleyi</td>
<td>3.8</td>
<td>$3.1 \times 10^{-11}$</td>
<td></td>
<td>0.023</td>
</tr>
<tr>
<td>Chaetoceros protuberans</td>
<td>26.5</td>
<td>$1.1 \times 10^{-9}$</td>
<td></td>
<td>0.021</td>
</tr>
<tr>
<td>Privoznik et al. 1978</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella pyrenoidosa</td>
<td>1.78</td>
<td>9,314</td>
<td>$1.85 \times 10^{-10}$</td>
<td>0.0043</td>
</tr>
<tr>
<td></td>
<td>1.84</td>
<td>6,563</td>
<td>$1.36 \times 10^{-10}$</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td>1.66</td>
<td>4,851</td>
<td>$0.86 \times 10^{-10}$</td>
<td>0.0076</td>
</tr>
<tr>
<td></td>
<td>1.60</td>
<td>4,176</td>
<td>$0.67 \times 10^{-10}$</td>
<td>0.0098</td>
</tr>
<tr>
<td></td>
<td>1.58</td>
<td>4,709</td>
<td>$0.73 \times 10^{-10}$</td>
<td>0.0089</td>
</tr>
<tr>
<td></td>
<td>1.68</td>
<td>5,430</td>
<td>$0.75 \times 10^{-10}$</td>
<td>0.0095</td>
</tr>
<tr>
<td>Our data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cricosphaera carterae</td>
<td>10</td>
<td>300.6</td>
<td>$1.20 \times 10^{-9}$</td>
<td>0.0113</td>
</tr>
<tr>
<td></td>
<td>13.5</td>
<td>38.5</td>
<td>$4.65 \times 10^{-9}$</td>
<td>0.0070</td>
</tr>
<tr>
<td></td>
<td>11.3</td>
<td>120.3</td>
<td>$6.67 \times 10^{-9}$</td>
<td>0.0094</td>
</tr>
<tr>
<td>Thalassiosira eccentrica</td>
<td>32.3</td>
<td>41.2</td>
<td>$1.39 \times 10^{-8}$</td>
<td>0.0107</td>
</tr>
<tr>
<td>Scrippsiella trochoidea</td>
<td>19.7</td>
<td>67.7</td>
<td>$3.94 \times 10^{-9}$</td>
<td>0.0134</td>
</tr>
<tr>
<td>Nannochloris sp.</td>
<td>2.5</td>
<td>142.0</td>
<td>$1.30 \times 10^{-10}$</td>
<td>0.0147</td>
</tr>
<tr>
<td>Kiel harbor, sample A$\S$</td>
<td></td>
<td></td>
<td>52.2</td>
<td>0.0110</td>
</tr>
<tr>
<td>Kiel harbor, sample B$\S$</td>
<td></td>
<td></td>
<td>7.2</td>
<td>0.0174</td>
</tr>
</tbody>
</table>

$t$ Diameter of cell-volume equivalent sphere.
$\S$ In vivo specific absorption coefficient of cell suspension.
$\S$ For species composition see Maske and Haardt 1987.

Therefore $\rho'_{sw}$ of a suspension of cells with pigmented chloroplasts would be related to $\rho'$ of a suspension of homogeneous cells as follows:

$$\rho'_{sw} = 2r_ia^* = \rho'z^{-\gamma_0}(1 - R_0)^{-\gamma_0}.$$ (11)

In the case of $R_0 = 0$ (i.e. homogeneous cells), cell division (doubling of $z$) would enhance specific absorption. In the case of constant $z$, contraction of pigmented volume would lead to reduced specific absorption. In the data analysis we consider phytoplankton cells to be homogeneous, expecting that the calculated $\rho'$ values are smaller than those calculated with more realistic cell morphology.

Results

We consistently found absorption peaks at 675–677 nm for seawater samples. Absorption spectra of particles ($a_{part.}$) were calculated by subtracting the absorption spectra of suspension filtrate $a_w(\lambda)$ and $a_s(\lambda)$ from the sample suspension. These spectra yielded specific absorption coefficients at 675 nm (Table 2), with values between 0.007 and 0.017 m$^2$ (mg Chl a)$^{-1}$. Specific absorption coefficients of cultures varied between 0.007 and 0.013.

A series of dilutions from 75 to 300 mg Chl a m$^{-3}$ with one sample gave no indication that the specific absorption coefficient varied with sample concentration. Figure 3 represents the original data without baseline correction. Dominant features of the spectra are Chl c absorption at 630 and 590 nm and carotenoid absorption at 500 nm. Care was taken to remeasure and use as a reference the blank with water filtered from each subsample. This step is necessary because $a_{sp}$, the absorption coefficient of dissolved yellow substance, varied for our samples between 0.005 and 0.02 m$^{-1}$ at 675 nm.

The addition of nonabsorbing latex spheres (diam, 1 $\mu$m) to a sample resulted in only a slight, nearly wavelength-independent decrease in absorption. With very high concentrations of latex (up to 10 times the concentration of phytoplankton cells) the specific absorption was no more than 30% below the original value.

Absorption spectra obtained with the suspension-integrating sphere method, in par-
ticular those from Kiel harbor samples, show relatively high absorption in the blue. This absorption was not detected in cultures and can be shown to be of particulate detrital origin (Maske and Haardt 1987). Cultures of C. carterae and S. trochoidea were dark adapted; their specific absorption coefficients at 675 nm (0.009 and 0.013 respectively) were not much different from normally grown cultures.

Discussion

Specific in vivo absorption of pigmented phytoplankton shows a narrow peak at about 675 nm due to Chl a absorption. This peak is actually the result of various in vivo Chl a fractions with slightly different spectra (Prézelin 1981; Larkum and Barrett 1983). At this wavelength there is no information on their molecular absorbance. Therefore cellular pigment absorption used in this context describes a composite property.

At 675 nm, overlap with Chl b and Chl c is negligible and therefore we chose the in vivo absorption at this wavelength to verify the concept of a constant specific in vivo absorption coefficient of Chl a (cf. Morel and Bricaud 1981). However at the Soret band of absorption near 440 nm the absorption of chlorophyll is strongly masked by absorption of accessory pigments, and therefore this spectral band is unsuitable for investigating the absolute absorption properties of Chl a in vivo.

A search of the literature for specific absorption coefficients of phytoplankton at 675 nm produced various values (Maske and Haardt 1987). With few exceptions all of them are considerably higher than ours. There are two possible explanations: sample characteristics might be drastically different, or the methodology might not be comparable. With respect to the latter we suggest three possible mechanisms leading to increased specific absorption coefficients: particle interaction in concentrated samples, unknown angular distribution of radiance, and multiple reflection of light on opal glass and inside the cuvette resulting from total reflection.

Butler (1962) demonstrated interaction of absorbing substances with nonabsorbing, only scattering particles leading to longer effective pathlength of light within samples. Kiefer and SooHoo (1982) took this effect into consideration by correcting their data measured on filtered samples according to comparative measurements of suspensions by separate determination of transmittance and reflectance by means of an integrating sphere. There remains a degree of uncertainty in their data because their correction, assumed to be a constant factor and obtained for only a few samples, might change with the type of sample.

In samples of high turbidity the incident beam will be scattered and the resulting light field will reach a certain degree of diffuseness. Because the nature of the light field will change with length of the light path this effect is difficult to quantify. One option would be to supply a diffuse incident light beam, which would lead to twice the absorption in collimated light. This potential source of error motivated our measurements of suspensions at low concentrations, close to those found naturally. Kiefer et al. (1979) chose to measure their absorption coefficients at high concentrations, arguing that it provided a diffuse light field within the sample. A doubling of absorption coefficients in diffuse light was to be expected relative to collimated light (Kubelka 1947); therefore it should theoretically be justifiable to divide their values by two to make them comparable to other published data (cf. Maske and Haardt 1987). Doing so would result in values of 0.01–0.014 m² mg⁻¹, close to our average values.

Measured absorption can be increased by the use of opal glass between sample and detector in the case of suspensions in small cuvettes. Because opal glass shows typical diffuse reflectance (Lambert reflector) of 60–80%, considerable light re-enters the cuvette. Even if the incident beam initially was collimated, the reflected light is diffuse because of the nature of the opal glass. As a result light will traverse the sample within the cuvette several times because of total reflection on the outside of the glass walls, especially at the front where the beam enters the cuvette. The percentage of reflected light finding its way to the detector will be strongly attenuated due to multiple passage. The above mechanism might explain the higher
values of absorption coefficients measured by Bannister (1979), Morel and Bricaud (1981), Bricaud et al. (1983), and Bannister and Weidemann (1984).

Measurement within an integrating sphere at moderate concentrations can be expected to yield absorption coefficients for collimated light with a minimum of scatter and interaction. We conclude so from the fact that even the addition of latex at high concentrations led to <30% reduction in absorption (from scattering of light out of the cuvette sides resulting in a decreased effective pathlength of the measuring beam within the cuvette). Because of their small size and high refractive index (ca. 1.2) relative to water, scattering of these latex spheres is generally much higher than by phytoplankton. The fact that a dilution series of C. carterae with concentrations between 300 and 75 mg Chl a m\(^{-3}\) produced no change in specific absorption coefficients indicates that our sample concentrations were well below those leading to possible scatter-induced artifacts.

Pigment extracts in acetone (in vitro) have a specific optical density of 87.67 liter \(\mu g^{-1}\), at a pathlength of 1 cm (Jeffrey and Humphrey 1975). This value can be transformed to a specific absorption coefficient of 0.0202 m\(^2\) (mg Chl a\(^{-1}\)). The specific absorption can vary (±15%) depending on the polarity and refractive index of the solvent (Seeley and Jensen 1965). The pigment concentrated in chloroplasts has an average specific coefficient of in vivo absorption of 0.011 m\(^2\) (mg Chl a\(^{-1}\)) (Table 2). One explanation for the different absorption coefficients in vivo and in vitro could be a variable coefficient of molecular absorption, which seems possible when the differences in peak absorbance wavelengths are considered. Even within the chloroplast various Chl a fractions show distinct spectral behavior, but there is no information on variations in specific molecular absorption in vivo. Another explanation that has been shown to account for the magnitude of the difference in absorption of phytoplankton in vivo and in vitro is based on the packaging or sieve effect (Duy sens 1956; Rabinowitch 1956), according to the varying optical efficiency factor \(Q_{abs}\) (cf. theoretical considerations). The increase of absorption with diminishing particle size was demonstrated very convincingly by Das et al. (1967) who sonicated a suspension of Chlorella culture. It resulted in absorption coefficients of the red peak increasing with sonicating time until they were close to absorption coefficients in a methanol extract. The theoretical and practical consequence of the packaging effect with respect to phytoplankton was discussed by Das et al. (1967), Kirk (1975), and Morel and Bricaud (1981).

With knowledge of optical density and geometry of cells, we can calculate the decrease in absorption when the pigment is concentrated homogeneously in particles (Hulst 1957; cf. theoretical considerations above), assuming an as yet unknown value of \(a^{*}_{chl}\), the cellular, in vivo specific absorption of Chl a. As a first approximation we assumed \(a^{*}_{chl}\) to be 0.02 m\(^2\) (mg Chl a\(^{-1}\)) following the Bricaud et al. (1983) argument that the in vivo specific absorption of Chl a might be close to the in vitro value in acetone. This assumption is supported by the result of Das et al. (1967) that a sonicated culture of Chlorella has absorption coefficients slightly less than those in methanol extract. Unfortunately they gave no values of specific absorption coefficients or of particle geometry. Nevertheless one can assume that pigments in the sonicated sample were distributed homogeneously enough to maximize absorption. Kirk (1975) calculated a specific absorption coefficient in vivo of 0.0237 and 0.0251 m\(^2\) (mg Chl a\(^{-1}\)) at 675 nm from literature data.

Our experimental data obtained with the suspension-integrating sphere are plotted in Fig. 4 as normalized specific absorption coefficients vs. \(\rho' = da\). In addition Fig. 4 shows the theoretically expected function based on assumptions of sphericity of algal cells and homogeneity of pigments within the cells (Eq. 10). A similar graph was presented by Duy sens (1956, figure 1). In the calculation of our data points the cell diameter (Table 2) was taken to be the diameter of spheres with volumes equivalent to the actual cells. All data points lie significantly beneath the expected values. This discrepancy might be
due to an overestimation of the specific cellular in vivo absorption coefficient of Chl a. A more likely explanation is that the values of \( p' \) are strongly underestimated because of the assumed homogeneity of the phytoplankton cells. Heterogeneity of pigment distribution in the cells due to chloroplast structures could be included in calculating \( p' \) (Eq. 11), leading to higher values in Fig. 4 compared to pigments distributed homogeneously within the cells. A decrease of the diameter of the pigmented cell volume by half would increase \( p' \) fourfold. Unfortunately the necessary morphological information for our data was not available, thus preventing verification of our hypothesis. But, in general, experimental data are always expected to lie below the theoretical results (Eq. 10) because of oversimplified assumptions on which the theory is based, e.g. homogeneity of cells.

We included in Fig. 4 those literature data points supplying the necessary information for the calculation, although the methods are not strictly comparable to ours. Privoznik et al. (1978) and Morel and Bricaud (1981) used transmission measurements of suspensions where the light reflected and that scattered beyond the angle of acceptance of the detector are not taken into account, thus presumably overestimating absorption. We modified the absorption data of Morel and Bricaud (1981) by subtracting a baseline—a straight line between data points at 720 nm and the minimum at about 550 nm. This step was justified by Bricaud et al. (1983), who treated data obtained with the same optical setup in such a manner. Despite the baseline subtraction, the data of Morel and Bricaud (1981) still exceed the predicted relationship. One likely reason, as pointed out above, would be the enhancement by opal glass placed between sample and detector. Another possible reason for overestimated absorption coefficients was suggested by Kiefer et al. (1979). High cell density of samples may lead to increased diffuseness of radiance within the sample. The normalized projected area of particles, \( A_T \), might give a guide for comparison. For the samples of Morel and Bricaud (1981) the \( A_T \) values are between 0.2 and 0.6, whereas those of Kiefer et al. (1979) were between 0.9 and 3.4. Kiefer et al. assumed completely diffuse light conditions in their sample and hence expected absorption coefficients twice those in collimated light.

The data of Privoznik et al. (1978) were taken with the sample directly in front of the photomultiplier. This geometry and low reflectance of the photomultiplier window constitute a close approximation of transmission measurements, inherently yielding slightly higher values than absorption coefficients for typical phytoplankton samples. Because their samples had very high cell concentrations they reached a normalized projected area of particles of about five in their 1-cm cuvette (Privoznik and Incropera 1978). According to Kiefer et al. (1979), light conditions in the cuvette should have been completely diffuse with a doubling of absorption coefficients. In fact their specific absorption coefficients seem too low relative to the calculated \( p' \) values (Fig. 4).

The specific absorption coefficients for 675 nm of Privoznik et al. (1978) might be slightly underestimated for two reasons: they measured absorption in vivo not at its maximum but at 670 and 680 nm, resulting in an underestimation of about 5%. The specific absorption coefficients for in vitro Chl a were 8% higher than those used for our calculation, and no sample turbidity was subtracted (Privoznik and Incropera 1978). Because it leads to overestimated in vitro Chl a concentrations, specific in vivo absorption coefficients would be underestimated by about 10%. Neither Privoznik and Incropera (1978) nor Privoznik et al. (1978) indicate how the electronic particle counter that determined cell size was calibrated. Faulty calibration of cell size cannot be excluded. One indication that either cell size or pigment methodology might have been faulty is the higher than expected intracellular chlorophyll concentration that can be calculated from their data (Bannister pers. comm.).

Morel and Bricaud (1981) argued that the bimodal distribution of cell size in the cultures of Privoznik et al. (1978) might have led to an underestimation of \( p' \). Our calculation for culture No. 2 (Privoznik et al.
based on the reported size-frequency distribution, actually does not support this explanation for the low specific absorption coefficients. Thus their unmodified values should be accepted.

Another species-specific optical parameter is the cell wall material. Species used (Table 2, Fig. 4) represent the three typical wall materials: calcium carbonate, silicate, and carbohydrate. It might be expected that reflection within the single cell would result in significant absorption enhancement due to increased pathlength, but apparently cell wall material has no influence on specific absorption (Fig. 3).

Kirk (1975), Morel and Bricaud (1981), and Kirk (1983) already pointed out a host of autecological consequences resulting from the packaging effect. The inherent variability (more than a factor of two in our data) of the absolute specific absorption coefficients of phytoplankton suspensions due to size, form, and pigment content of cells as well as internal cell architecture is of obvious importance in evaluating photosynthetic performance. Particularly noteworthy is the fact that the flattening of absorption spectra and variations in the ratio of absorption at 440/675 nm is a result of the packaging effect (Das et al. 1967; Morel and Bricaud 1981, cf. their figure 2). Shade-adapted cells of higher pigment content or smaller cells of similar pigment content would have lower coefficients of specific absorption; thus they have flatter spectra with lower absorption ratios at 440/675 and 675/600 nm. This contention is supported by the results of SooHoo et al. (1986), although the change in ratio was only on the order of 10%.

Das et al. (1967) argued that with a normalized projected area of particles >1 the spectral deformation due to the packaging effect should vanish. This hypothesis is based on geometrical considerations and is misleading since scattering is not considered. In our data we could show that even at a normalized projected area of particles of 1.74 (Fig. 2, 300 mg Chl a m⁻² = 1.74 norm. proj. area), there is no increase in specific absorption with increasing concentration.

It might be expected that under low light conditions algal cells would enhance their specific absorption. This enhancement could be achieved by larger chloroplast size—an adaptive mechanism reported for some algae (table 7; Larkum and Barrett 1983). The increase of cellular Chl a concentration would actually lead to lower coefficients of specific absorption. We grew S. trochoidea and C. carterae cultures under low light conditions (5 × 10⁻⁶ mol quanta m⁻² s⁻¹; 12:12 L/D). They showed no significantly different specific absorption coefficient for Chl a than the cultures of the same species grown under high light (150 × 10⁻⁶ mol quanta m⁻² s⁻¹; 12:12 L/D), although at least C. carterae had increased its Chl a concentration per cell by a factor of 1.5–5 (Table 2) under low light conditions. One explanation might be that the shade-adapted cell had increased chloroplast dimensions.

The above discussion was based on the assumption that phytoplankton cells behaved optically like spheres of equivalent volume (Eq. 2). If the higher average geometrical cross section of nonspherical cells (factor F, Eq. 2) were included, the theoretical specific absorption value in Fig. 4 would be higher, leading to a greater difference between data and theory. If the chloroplast dimensions were chosen as the relevant pigment container, then $p'$ would increase (Eq. 11), leading to better agreement of data and theory. The latter approach would need to take into account that chloroplasts are not randomly distributed within the cell but have a tendency to abut the inside of the cell wall.

Conclusions

Further experimental investigations should aim at the influence of inhomogeneity within the cells and the effects of thylakoid scattering. The latter is ignored in the theoretical approach of Duysens (1956) and the approximations by Hulst (1957) as used in this investigation, but it might lead to considerably enhanced absorption (Kubelka 1947).

References


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