A METHOD OF DETERMINING EXTINCTION COEFFICIENTS FOR NATIVE PIGMENTS IN CELL SUSPENSIONS

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It is difficult to determine the true absorption coefficients of pigments in cells (erythrocytes, algae, bacteria, yeasts) without a knowledge of the optical thickness τ because the optical parameters are often altered when they are transferred to solution, when this is possible at all.

We have shown [1-4] that the absorption by a suspension is determined by $\tau = kd$, where k is extinction coefficient and d is particle diameter. One factor relating the absorption to τ is $\gamma = D_S/D$, where D_S is the optical density of the cell suspension and D is the true optical density of the pigment.

The $\gamma(\tau)$ curves for cells show that the following approximation applies up to $\tau = 2.0$ with an error not exceeding 0.005-0.01:

$$\gamma = 10^{-\alpha\tau+\beta\tau^2} = 10^{-0.37\tau+0.052\tau^2} \quad (1)$$

Here τ is some integral average optical thickness incorporating the various aspects of the native state of the pigments and particles (aggregation, concentration, molecular interaction), as well as the shape and size of the cells.

D_S is the quantity most readily measured and is

$$D_S = \gamma \cdot D = n\gamma\tau = n\tau_r \quad (2)$$

where

$$\tau_r = \gamma\tau \quad (3)$$

is the apparent (recorded) optical thickness of the cells and n is a coefficient of proportionality. We see from (2) that D_S is linearly related to τ_r, so it is best to express γ not as a function of τ but of τ_r (Fig. 1). The numerical values of γ and τ_r have been derived via (1) and (3). Figure 1 indicates that the absorption spectra of cell suspensions allow one to draw up $D(\lambda)$ (true optical density of the native pigments) if we know γ for some one wavelength λ_1 (base value γ_1).

The linear relation of D_S to τ_r in (2) allows us to express $\tau_{\lambda_i,r}$ for any λ. If we know D_{S,λ_1}, D_{S,λ_i}, and τ_{λ_1}, we have

$$\tau_{\lambda_i,\,r} = \tau_{\lambda_1,\,r}\,\frac{D_{S,\,\lambda_i}}{D_{S,\,\lambda_1}}. \quad (4)$$

From Fig. 1 we get γ_{λ_i}, and then

$$\tau_{\lambda_i} = \frac{\tau_{\lambda_i,\,r}}{\gamma_{\lambda_i}} \quad (3a)$$

and

$$D_{\lambda_i} = \frac{D_{S\ \lambda_i}}{\gamma_{\lambda_i}} \quad (2a)$$

give the true optical thicknesses τ_{λ_i} and optical densities D_{λ_i} of the solutions of the native pigments.

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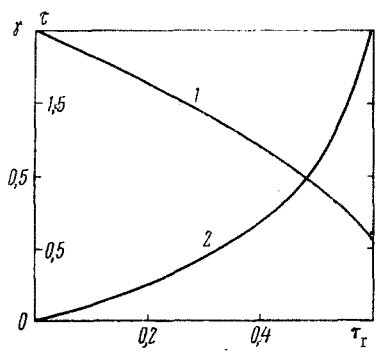

Fig. 1

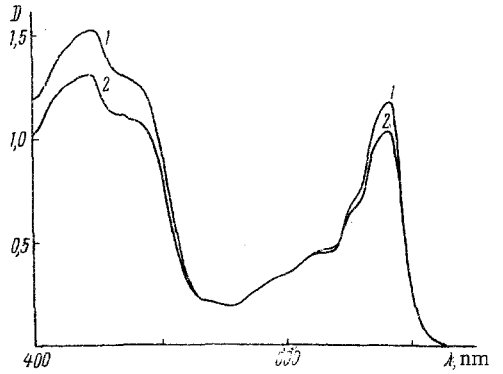

Fig. 2

Fig. 1. The τ_r dependence of: 1) γ; 2) τ.

Fig. 2. Absorption spectra of Chlorella suspended in: 1) distilled water; 2) 42% glucose solution.

There are great difficulties in measuring γ even for one wavelength without disrupting the cells. Sometimes microspectrophotometry can be used: in other cases, one can use $\gamma(\tau)$ for a cell suspension, where τ is varied by means of some external factor that leaves the optical parameters of the native pigments unaltered. For instance, τ can be varied by use of solutions containing various concentrations of salts, glucose, glycerol, etc.

Of course, the shape of the cell may affect the relation of τ to tonic strength, but this relationship takes a simple analytical form for particles of regular shape. We consider uniform spherical particles as an example and relate τ to the size via the ratio of the τ before and after size change.

We assume that the particles have a constant pigment content: then

$$\frac{1}{6}\pi d_0^3 c_0 = \frac{1}{6}\pi d^3 c \tag{5}$$

gives

$$\frac{c_0 d_0}{cd} = \left(\frac{d}{d_0}\right)^2. \tag{6}$$

Here subscript zero denotes the initial state.

We put $\tau = k'cd$ (k' is the specific extinction coefficient) to get

$$\frac{\tau}{\tau_0} = \frac{k'cd}{k'c_0 d_0} = \left(\frac{d_0}{d}\right)^2 = \delta, \tag{7}$$

i.e., τ/τ_0 is not dependent on the spectral extinction coefficient and is governed solely by the geometrical size change. Equation (7) applies also to other particles whose shape does not change, e.g., Chlorella cells.

We assume that (7) applies throughout the spectral region and use $\gamma(\tau)$ as in (1) to find the true averaged optical thicknesses of the cells and the true optical densities of the native pigments. We have to solve equations relating D_S and D for some two wavelengths:

$$\begin{aligned}
D^0_{1,s} &= 10^{-\alpha\tau_1^0+\beta(\tau_1^0)^2}\cdot D_1,\\
D_{1,s} &= 10^{-\alpha\delta\tau_1^0+\beta(\delta\tau_1^0)^2}\cdot D_1,\\
D^0_{2,s} &= 10^{-\alpha\tau_2^0+\beta(\tau_2^0)^2}\cdot D_2,\\
D_{2,s} &= 10^{-\alpha\delta\tau_2^0+\beta(\delta\tau_2^0)^2}\cdot D_2.
\end{aligned} \tag{8}$$

If $\tau = 0.3$ is not exceeded, we can take only the first term in the exponent on the right in (1), i.e., we have

$$\gamma = 10^{-\alpha\tau} = 10^{-0.36\tau}. \tag{9}$$

Then (8) becomes

$$\begin{aligned} D^0_{1,s} &= 10^{-\alpha\tau^0_1}\cdot D_1, \\ D_{1,s} &= 10^{-\alpha\delta\tau^0_1}\cdot D_1, \\ D^0_{2,s} &= 10^{-\alpha\tau^0_2}\cdot D_2, \\ D_{2,s} &= 10^{-\alpha\delta\tau^0_2}\cdot D_2. \end{aligned} \tag{10}$$

We solve (10) with (2) to get

$$\tau^0_1 = \frac{\lg\dfrac{D^0_{2,s}}{D^0_{1,s}} + \lg\left(\lg\dfrac{D^0_{1,s}}{D_{1,s}} \Big/ \lg\dfrac{D^0_{2,s}}{D_{2,s}}\right)}{\alpha\cdot\left(1 - \lg\dfrac{D^0_{2,s}}{D_{2,s}} \Big/ \lg\dfrac{D^0_{1,s}}{D_{1,s}}\right)}. \tag{11}$$

If we know the relation of τ_0 to τ, e.g., via (7) for spherical particles, we can determine the optical thickness in another way simply by solving (9) in terms of D_S for one wavelength before and after the size change:

$$\begin{aligned} D^0_S &= \gamma(\tau_0)\cdot D = 10^{-\alpha\tau_0}\cdot D, \\ D_S &= \gamma(\tau)\cdot D = 10^{-\alpha\tau}\cdot D \end{aligned} \tag{12}$$

or

$$D^0_S = \gamma(\tau_0)\cdot D = 10^{-\alpha\tau_0}\cdot D,\quad D_S = \gamma(\delta\tau_0)\cdot D = 10^{-\alpha\delta\tau_0}\cdot D. \tag{13}$$

Finally we find

$$\frac{D^0_S}{D_S} = \frac{\gamma(\tau_0)}{\gamma(\delta\tau_0)} = b. \tag{14}$$

Consider the ratio of the optical densities for cell suspensions to which (1) and (7) apply. We substitute for $\gamma(\tau_0)$ and $\gamma(\delta\tau_0)$ from (1) into (14) to get

$$\frac{D^0_S}{D_S} = 10^{-\alpha(1-\delta)\tau_0+\beta(1-\delta^2)\tau_0^2} = b, \tag{14a}$$

so

$$\tau_0 = \frac{(1-\delta)\alpha + \sqrt{(1-\delta)^2\alpha^2 + 4\beta(1-\delta^2)\lg b}}{2\beta(1-\delta^2)}. \tag{15}$$

From (1), (2), (4), and Fig. 1 we get γ_{λ_1}, $\tau_{\lambda_1,r}$, $\tau_{\lambda_i,r}$, and γ_{λ_i}; from D_S and γ we get the true optical densities D_λ, and from

$$k_\lambda = \frac{D_\lambda}{\lambda_\lambda\cdot l} \tag{15a}$$

we get the true extinction coefficients of the native pigments (l is the thickness of the layer of suspension).

As an example we consider the specific extinction coefficient for the native chlorophyll of algae at the red peak 680 nm.

Figure 2 shows absorption spectra of Chlorella (single-celled algae) in distilled water and in 42% glucose solution. The band positions do not alter, but the numerical values of the absorption coefficients differ. Direct microscope measurements gave $\delta = 1.55$; then (1) and (15) give $\tau^0_{680} = 0.3$ and $\gamma_{680} = 0.782$. We substitute for $D_{680,S}$, c, and l in

$$k'_{680} = \frac{D_{680,\,s}}{\gamma_{680}\cdot c\cdot l}, \tag{16}$$

to get for $\lambda = 680$ nm that:

$$k'_{680} = 89000\ \text{cm}^3/\text{g}. \tag{17}$$

This method for k' allows one to derive fairly accurately (to 5%) the true absorption of native pigments and the concentrations in cells without disruption.

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