THE FLATTENING OF THE ABSORPTION SPECTRUM OF SUSPENSIONS, AS COMPARED TO THAT OF SOLUTIONS

by

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INTRODUCTION

Biological systems may contain particles, such as blood cells, chloroplasts or nuclei, which possess a marked absorption in the visible or near ultraviolet, where the bulk of the cell material does not absorb.

It will be shown in this paper that the absorption spectrum of such a system or, more generally, of any suspension of strongly absorbing particles, even if properly corrected for scattering, must appear flattened compared with that of a solution containing the same molecules in homogeneous dispersion; the higher the absorption of the individual particles, the stronger the flattening. As far as we know, this distortion of the spectrum has not been taken into account in previous investigations; in consequence, no satisfactory analysis of the absorption spectrum of a suspension of strongly absorbing particles has been as yet carried out. In this paper a method will be described, which makes possible the evaluation of this distortion and the determination of the optical density of individual particles.

These optical densities are of importance, for example, for the analysis of the empirical absorption spectra of suspensions in terms of the contributions from several colored components which may be present in them—an analysis necessary, *e.g.*, for an evaluation of the relative photochemical efficiencies of light quanta absorbed by these pigments.

A well-known case is that of the relative efficiencies of the chloroplast pigments in photosynthesis. The same data are also required for the determination of the amounts of the several pigments, present in a suspension, from the absorption spectra.

Suspension of cubical particles

We shall first consider the absorption spectrum of a simple model system, consisting of a suspension of homogeneous cubical particles in a rectangular vessel with an illuminated area of 1 cm^2.

One of the walls of the cubical particles is perpendicular to the measuring beam. (Later, we shall derive equations for particles of arbitrary shape.)

The following notations are used:

d is the edge of a cube,

h is the depth of the vessel in cm, which is equal to the volume in cm^3;

$a_{sol} = \ln I_0/I$ is the optical density of the solution;

T_p is the transmission of a single cube for a given λ (ratio of the intensities of the beam before and after passage through it),

$a_{sus} = \ln I_0/I$ is the optical density of the suspension,

p = number of layers of cubes which would be formed if all cubes were stacked and the cubes, forming an incomplete layer, squeezed flat.

In the solid layer formed in the last mentioned way, the pigment distribution is homogeneous; consequently, its absorption spectrum must be identical with that of the solution,

$$a_{sol} = p \ln 1/T_p \tag{1}$$

The assumption is made in the whole discussion that the absorption by a single molecule is *not* affected by interaction with other molecules in the particle.

In order to calculate the absorption spectrum of the suspension, we imagine the vessel to be divided in a regular array of cubic compartments, with a volume d^3. The probability, q, of finding a certain compartment occupied by a particle is equal to the ratio of the total volume of the particles, pd, and the volume of the vessel h; or $q = pd/h$. The uniform measuring beam with an area of 1 cm², passing through such an array of cubic compartments, may be thought of as being composed of $1/d^2$ light pencils, each traversing a separate column containing m $(= h/d)$ cubic compartments. The probability that k $(k \leq m)$ specific compartments in such a column each contain a particle (while the $m - k$ remaining compartments of the column contain none) is $q^k (1 - q)^{m-k}$. (It is assumed that the particles do not attract or repulse each other, since then the presence of a particle in one compartment would change the probability of finding a particle in an adjacent one.)

The number of ways we can choose k compartments from the m compartments in a column is: $m!/[k!(m - k)!]$. Thus, the probability for finding a column containing k particles is: $P_k = \{m!/[k!(m-k)!]\}\, q^k(1-q)^{m-k}$. If I_0 is the energy of the monochromatic beam incident on the whole vessel, then the energy incident on all the columns containing k particles is I_0P_k, and the energy transmitted by these columns is $I_0P_kT^k{}_p$, where T_p is the transmission of a single particle. The total transmission of the vessel, T, is found by summing the last quantities over all k's and dividing by I_0.

$$T = \sum_{k=m}^{k=0} \left\{ m!/[k!\,(m - k!)] \right\} (qT_p)^k \,(1 - q)^{(m - k)}.$$

The right hand side of this equation is Newton's binominal expansion of

$$[q\,T_p + (1 - q)]^m = [1 - q\,(1 - T_p)]^m.$$

The optical density of the suspension a_{sus} is $\ln (1/T)$.

$$a_{sus} = - m \ln [1 - q\,(1 - T_p)] \tag{2}$$

According to RABINOWITCH (private communication), JACOBS has obtained a similar equation.

If the whole vessel is filled up with cubes then $q = 1$ and $a_{sus} = m \ln(1/T_p)$, an equation which is obtained directly by application of Beer's law.

If $q(1-T_p) \ll 1$, we have in first approximation:

$$a_{sus} = mq(1 - T_p), \text{ or, since } mq = p,$$
$$a_{sus} = p(1 - T_p) \tag{3}$$

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We derived eq. (3) previously in a simpler way, assuming the Poisson distribution for the particles. As pointed out in RABINOWITCH[2], the condition for the validity of eq. (3) is that the total volume of the particles is small compared to the volume of the suspension, or $q \ll 1$.

In the present paper, we derived first the more general eq. (2), in order to be able to estimate the error made when applying eq. (3). From the development of eq. (2) into a series, it follows that for $q \leq 0.1$

$$\{(\alpha'_{\text{sus}} - \alpha_{\text{sus}})/\alpha_{\text{sus}}\} < 0.6\,q\,(1 - T_p) < 0.6\,q \tag{4}$$

In other words, the fractional deviation of the calculated optical density of the particle from its true absorption is smaller than 0.6 × (total volume of particle)/(volume of vessel). For instance, a suspension of *Chlorella* cells, containing an equivalent of four complete layers of cells, (such a suspension has an appreciable optical density in the maxima of the absorptionspectrum) has a q value of about $4 \times 5/1000 = 0.02$, assuming the cells have a diameter of 5μ and are suspended in a vessel of 1 cm depth. The absorption value calculated by means of (3) differs less than $0.6 \times 0.02 = 0.12$ percent from the true value.

Comparison of eq. (1) and (3) shows that the optical density of a solution of molecules, α_{sol}, is different from the density, α_{sus}, of a suspension of particles containing the same total number of molecules.

The quotient $\alpha_{\text{sus}}/\alpha_{\text{sol}} = (1 - T_p)/\ln(1/T_p)$ is independent of the concentration; it approaches zero if T_p becomes small. It approaches unity if T_p nears unity, that is if the particles become transparent. In other words the "flattening" increases as the absorption by the individual particles increases; it disappears when this absorption becomes small.

Suspension of particles of arbitrary shape

We shall use another method, an approximative one, in the following calculations. It is based on the assumption that Beer's law is valid for suspensions, in other words that the optical density, $\ln I_0/I$, of a suspension with N particles is N times that of a "suspension" of one particle.

(The validity of Beer's law for suspensions has been postulated before, and has been verified experimentally for certain types of suspensions (*cf.* for instance[3]); however, it will be shown below that the law is only valid for dilute and moderately concentrated suspensions.)

RABINOWITCH[2] has applied Beer's law to a suspension of cubes and obtained in this way equation (3). We shall give here a derivation holding for suspensions of particles of any shape, size and oreintation. To begin with, we shall assume that all particles have the same size and orientation; this restriction will be dropped later. The following notations will be used:

a_p is the area of the projection of a particle on a plane perpendicular to the light beam,

T_p, the transmission of a particle, defined as the total energy flux per second passing the particle, divided by the energy flux incident on it.

N is the number of particles in the suspension.

We calculate first the optical density, α'_{sus}, of a "suspension" of one particle of arbitrary shape.

$$\alpha'_{\text{sus}} = \ln I/I_0 = \ln [1 - a'_p (1 - T_p)]$$

If the area a_p is small compared with the area of the measuring beam, ($a_p \ll 1$), then the preceding equation is approximated by:

$$\alpha'_{\text{sus}} = a_p (1 - T_p)$$

This approximation holds within 0.1% if the particles are 0.3 mm in diameter and even better for smaller particles. If "Beer's law" is valid for a suspension of N particles, then

$$\alpha_{\text{sus}} = N\, a_p (1 - T_p) \tag{5}$$

Na_p is the total area of the N particles facing the light. Since the area of the vessel is 1 cm², the average number, p, of particles hit by a very thin beam is

$$p = N\, a_p \tag{6}$$

Substitution in (5) gives:

$$\alpha_{\text{sus}} = p (1 - T_p) \tag{7}$$

The quantity α_{sus} will now be expressed in terms of the shape, orientation and optical density of the particles.

The total volume of the particles is $N\, v_p$, where v_p is the volume of one particle. If l is a certain dimension of the particle, then

$$v_p = c_1 l^3 \tag{8}$$

Here c_1 is a constant which can be calculated from the shape of the particle. If all particles were squeezed against the wall of the vessel which faces the light, a homogeneous plane parallel layer of thickness $N\, v_p$ cm. would result. The absorption per unit of depth in this layer we call γ. The absorption of this layer is equal to the absorption of the solution, α_{sol}. Thus

$$\alpha_{\text{sol}} = N\, v_p \gamma = N\, c_1 l^3 \gamma \tag{9}$$

We introduce the quantity

$$\alpha_p = l\gamma \tag{10}$$

—the optical density corresponding to a path l through a particle. Since the projected area a_p of the particle is proportional to l^2, we have

$$a_p = c_2 l^2, \tag{11}$$

where c_2 is a constant depending on the shape and orientation of the particle. The transmission T_p, is a function of shape and orientation of the particle and of α_p.

$$T_p = F_s (\alpha_p). \tag{12}$$

Eliminating l, γ, and $N\, a_p$ from equations (6), (9), and (11), we obtain:

$$\alpha_{\text{sol}} = p\, (c_1/c_2) \alpha_p. \tag{13}$$

The quotient, Q_A, of the optical densities of the suspension and of the solution, which is a measure of the "flattening" effect, is, (*cf.* eq. 7 and 3):

$$Q_A = \alpha_{\text{sus}}/\alpha_{\text{sol}} = (1 - T_p)\, c_2/(c_1 \alpha_p). \tag{14}$$

Since p does not occur in this expression, Q_A is independent of the number of particles; in other words the flattening is the same for dilute as well as for concentrated suspensions.

If we assume that the shape and orientation of the particles are known, then c_1, c_2 and the functional relation (12), $T_p\ (a_p)$, can all be calculated. If, now, Q_A is determined experimentally at one wavelength λ_1, then the optical density of a single particle, $a_p\ (\lambda_1)$, can be calculated from eq. (14). Since Q_A is the quotient of two extinctions, it can be determined by means of a spectrophotometer.

From equation (7) we can find p, since $a_{\text{sus}}\ (\lambda_1)$ and $T_p\ (\lambda_1)$ are known. For all wavelengths λ, for which only $a_{\text{sus}}\ (\lambda_1)$ has been determined, T_p can be calculated from eq. (7), then a_p from equation (12) and a_{sol} from equation (13). The size of the particle can be calculated from the relation (10), provided we know γ.

If the ratio of the numbers of pigment molecules present in the solution and in the suspension is unknown, we cannot calculate Q_A by means of eq. (14). However, if Q_{sol} and $Q^1{}_{\text{sus}}$ (the superscript is to indicate possible differences in absolute amounts of pigments in solution and in suspension) are known at two wavelengths, λ_1 and λ_2, we can calculate $a_p\ (\lambda_1)$ and $a_p\ (\lambda_2)$ from the two equations

$$Q_{\text{sol}} = \alpha_{\text{sol}}\ (\lambda_1)/\alpha_{\text{sol}}\ (\lambda_2) = \alpha_p\ (\lambda_1)/\alpha_p\ (\lambda_2) \tag{15}$$

and

$$Q'_{\text{sus}} = \alpha'_{\text{sus}}\ (\lambda_1)/\alpha'_{\text{sus}}\ (\lambda_2) = \{1 - T_p\ [\alpha_p\ (\lambda_1)]\}/\{1 - T_p\ [\alpha_p\ (\lambda_2)]\} \tag{16}$$

This procedure is of particular advantage if it is difficult to disperse quantitatively into a molecular solution the pigments contained in the particles.

We now drop the restriction that all particles have the same size and orientation. The suspension can then be considered as a mixture of suspensions of particles, each suspension containing particles of the same size and orientation.

Instead of eq. (7), we get an equation in which the right hand side is averaged over all sizes and orientations. Q_A takes the form:

$$Q_A = \overline{p(1 - T_p)}\Big/\overline{(p(c_1/c_2)\alpha_p}$$

The line indicates the average of the function under it. The p's are proportional to the number of particles in the suspension, thus in this case, too, the flattening, Q_A, is independent of the concentration.

Validity of Beer's law for suspensions of particles of arbitrary shape

Beer's law is not strictly valid for a suspension of particles. This follows from the form of eq. (2), which gives the optical density of a suspension of oriented cubes as a function of their concentration. However, as long as the total volume of the cubes is small compared with the total volume of the suspension, equation (2) can be approximated by eq. (3), which does follow Beer's law (α = constant × q). As will be shown now, similar considerations apply to suspensions containing particles of arbitrary shape. Furthermore, the following derivation does not seem to require that the laws of geometrical optics obtain and, consequently, is valid also for very small particles.

If the number of particles per ml is n, a layer of thickness dx contains ndx particles. Beer's law requires that the light flux, $-dI$, absorbed in this layer, is proportional to the number of particles,

$$-dI = cI\ ndx \tag{17}$$

(I being the flux incident on the layer and c a constant)
The optical density of the suspension is:

$$\ln I_0/I_1 = cnd \tag{18}$$

where d is the depth of the vessel, and I_0 and I_1 are the fluxes entering and leaving the suspension.

Eq. (17) holds approximately if two conditions are fulfilled. First, the layer dx should not be thinner than a single particle; thus we must assume that

$$dx = bl$$

where b is a number slightly larger than unity and l is a dimension of the particle. Second, for the absorption in the layer to be proportional to the number of particles in it, ndx, the particles within a layer should not shade each other appreciably. This requires that the total area of the particles in the layer, $ndxc_2l^2$, (*cf.* eq. (11)) times the average percentage absorption, $1 - T_p$, be small compared to the total area of the layer:

$$ndxc_2l^2 (1 - T_p) \ll 1$$

Eliminating dx from the two preceding formulas, we obtain:

$$nc_2bl^3 (1 - T_p) \ll 1$$

This can be written as:

$$nc_1l^3 (1 - T_p) \ll c_1/(c_2b)$$

Since c_1, c_2 and b are of the order of unity, and nc_1l^3 is equal to the total volume, q, of the particles per ml, the inequality may be simplified

$$q (1 - T_p) \ll 1$$

In other words, the condition which must be fulfilled for Beer's law to obtain for a suspension of particles. is that the total volume of the particles in 1 ml multiplied by the fraction of light absorbed by a single particle, should be small compared to the volume in which the particles are suspended. For strongly absorbing particles, $(1 - T_p) \approx 1$, the fraction of the volume occupied by the particles should be small.

APPLICATIONS

Cubical particles, range of applicability of equations

For a suspension of cubes with two sides perpendicular to the measuring beam, we find, by putting the dimension l equal to the edge of the cube $c_1 = 1$, $c_2 = 1$, and $T_p = \exp(-\alpha_p)$. Substituting $c_1 = 1$ and $c_2 = 1$ in the general equations (13), (7) and (14), we obtain again:

$$\alpha_{\text{sol}} = p\,\alpha_p \text{ and } \alpha_{\text{sus}} = p\,(1 - T_p) \text{ and}$$

$$Q_A = \alpha_{\text{sus}}/\alpha_{\text{sol}} = (1 - T_p)/\alpha_p.$$

The equation $\alpha_{\text{sus}} = p(1 - T_p)$ is identical with eq. (3), which we derived before as an approximation of the exact eq. (2). The maximum fractional deviation was given as $0.6\, q(1 - T_p)$, eq. (4), where q is the total volume of the cubes divided by that of the suspension. This expression probably also holds approximately for other particles (such as spheres) whose shape is not too different from that of a cube (*i.e.* which extend more or less uniformly in all directions).

We can apply the approximate equations if we use a sufficiently dilute suspension. However, in order to be able to measure the absorption with good precision, the optical density should be at least 0.3. In practice, both conditions can be complied with, if the particles are not too large and possess an appreciable absorption. As an example, we take a suspension of particles 10μ in diameter, having a $T_p = 0.5$. If $p = 2$, then the approximate optical density $\log I_0/I$ of the suspension is $\alpha_{\text{sus}}/\ln 10 = 2 \times 0.5 \times 0.4 = 0.4$.

The total volume of the particles is $2 \times 10 \times 10^{-4}$ cm³. If the volume of the vessel is 1 cm³, then $q = 2 \cdot 10^{-3}$. The deviation, given by eq. (4) is $0.6 \times 2 \cdot 10^{-3} \times (1 - 0.5)$ or 0.06%.

Thus, even for such rather large particles, eq. (7) is a good approximation.

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In our first derivation of the equations for cubes, the assumption was used implicitly that a light beam with the cross section equal to that of the particles, follows the law of geometrical optics. This requires that the particle diameter be several times the wavelength of the measuring beam. Our derivation based on Beer's law makes no use of the laws of geometrical optics. This indicates that the equations obtained are also valid for particles not larger than the wavelength of light.

The equations derive for cubical particles are valid for any suspension of particles whose shape and orientation is such that every pencil of the measuring beam has the same length l to cover within the particle.

Suspensions of spherical particles

More important for practical applications than the equations for a suspension of oriented cubes are those for a suspension of identical spherical particles. For such a suspension, we shall derive the explicit equations, and construct a nomograph, permitting the determination of the absorption by a single particle from absorption measurements on a suspension and on the corresponding solution. We put the dimension l used in the above general equations equal to twice the diameter of a sphere: $l = 2r$. The optical density for a beam passing through the center of the sphere is $\alpha_p = 2r\gamma$. The area of the sphere facing the beam, is $a_p = \pi r^2 = (\pi/4\ (2r)^2$ or, *cf.* eq. (1), $c_2 = \pi/4$. The volume is $v = (4/3)\pi r^3 = (1/6)\pi(2r)^3$ or, *cf.* eq. (8), $c_1 = \pi/6$, and $c_1/c_2 = 2/3$. By substituting the constants in the equations of the preceding section, we obtain:

$$\alpha_{sus} = p\ (1 - T_p) \tag{18}$$

$$\alpha_{sol} = (2/3) p\ \alpha_p \tag{19}$$

$$Q_A = \alpha_{sus}/\alpha_{sol} = 3\ (1 - T_p)/(2\alpha_p) \tag{20}$$

We calculated the average transmission, T_p, by integrating over the sphere, using cylindrical coördinates. A line parallel to the measuring beam through the center of the sphere is used as the axis of the cylinders. The result of this integration is:

$$T_p = 2\ [1 - (1 + \alpha_p) \exp\ (-\alpha_p)]/\alpha_p{}^2. \tag{21}$$

Nomographs for suspension of identical spherical particles

The average transmission of the particles, calculated from eq. (21), is plotted in Fig. 1 as a function of α_p. The flattening, Q_A, given by eq. (20), is also plotted in this figure as a function of α_p.

The flattening increases with the absorption and is already appreciable at an average transmission T_p of 30%. For example, if, at a certain wavelength, the absorption of a suspension is one half that of a corresponding solution, then $Q_A = 0.50$

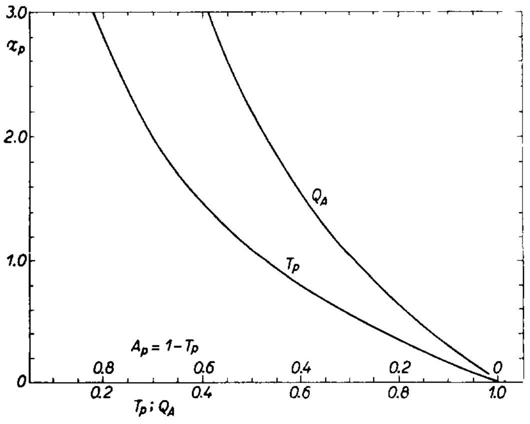

Fig. 1. The average transmission of a sphere, T_p, at a certain wavelength is plotted as a function of the optical density, $\alpha_p = \ln I_0/I$, through the center of the sphere. Also the ratio, Q_A, of the optical densities of a suspension of spheres and a corresponding solution is plotted as a function of α_p.

and we read, from the curve $Q_A = 0.5$ in Fig. 1, $a_p = 2.2$. The graph T_p in Fig. 1 gives the transmission of a particle 0.27 or 27%. The most precise readings are obtained at wavelengths where the flattening is most pronounced, that is, in the absorption maxima.

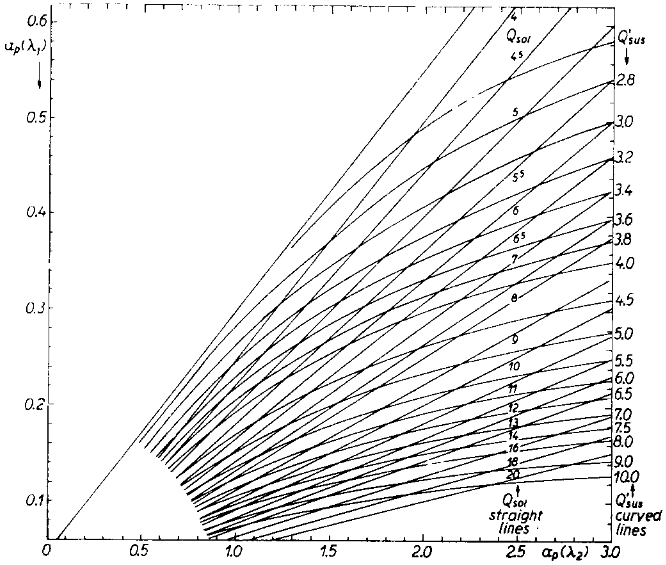

Fig. 2. Q'_{sus} is the ratio of extinction coefficients of a suspension of spheres at the wavelength λ_2 and λ_1. Q_{sol} is the quotient of the extinction values at λ_1 and λ_2 of a solution containing the same relative concentration of pigments as the particles. The intersection of the curve indicated by Q'_{sus} and the line indicated by Q_{sol}, gives the optical density values, $a_p(\lambda_1)$ and $a_p(\lambda_2)$, of the individual spheres.

If only an unknown fraction of the original pigment molecules can be obtained in solution, the procedure described in the preceding section is not applicable, since the flattening, Q_A, cannot be determined. However, the a_p's at these wavelengths may be derived from the equations (15) and (16), if we know Q'_{sus} and Q_{sol} at two wavelengths.

This may be done graphically by means of Fig. 2. Equation (15) is represented by a family of straight lines, each corresponding to a certain Q_{sus}; equation (16) by a family of curves, each corresponding to a certain Q'_{sol}. The intersection point of a certain line with a certain curve gives a_p (γ_1) and a_p (γ_2), corresponding to a certain pair of values Q. These values can be determined experimentally. Thus the optical densities of the particle at γ_1 and γ_2 can be read from the nomograph, if the optical densities of a suspension of these particles and of a pigment solution of arbitrary concentration are known at the wavelengths γ_1 and γ_2.

The curves given by equation (16) for certain values of Q_{sus} have been constructed as follows. We put $1 - T_p[a_p(\lambda_1)] = A$, and $1 - T_p[a_p(\lambda_2)] = B$, and made a table of values $[A, B]$ satisfying, for a given Q'_{sus}, the equation $Q'_{sus} = A/B$. Then the values of the a's, corresponding to these A's and B's were found by means of Fig. 1, and a table of pairs $[a_p(\lambda_1), a_p(\lambda_2)]$ corresponding to the pairs $[A, B]$ was prepared. These pairs are represented by points in the $a_p(\lambda_1)$, $a_p(\lambda_2)$ diagram. The curves corresponding to given Q'_{sus} values, were obtained by connecting these points.

Experimental procedure

The optical density of spherical perticles in a suspension can be determined by means of the figures in the preceding sections, if the densities of the suspension and of the corresponding solution are known at one or at two wavelengths, depending on whether the average pigment concentration is the same or not. The solution may be replaced by a suspension, in which the pigment is so finely dispersed that the absorption of each single particle is negligible. (Such a suspension must have the same absorption spectrum as a molecular solution.) For convenience we shall use the term "solution" also for this type of dispersions.

The dispersion of the pigments contained in the particles of the suspension may be accomplished by chemical or mechanical methods or by a combination of both. Mechanical methods are: grinding of precipitate, obtained *e.g.* by centrifugation; forcing the suspension through a small orifice; ultrasonic treatment. Chemical methods are: treatment with enzymes or chemicals which split the particles into smaller units or separate the pigment molecules from the carrier material.

In addition to the sharpening of the bands (the effect with which we are concerned) dispersion may also cause changes in the intrinsic absorption capacity of the pigment molecules. The absorption properties of the pigment molecules may be somewhat altered even by dispersion into smaller colloidal particles, since after this dispersion the pigment molecules will be in increased interaction with the molecules of the solvent and decreased interaction with each other. Greater changes may be expected to occur when the pigment is dispersed molecularly, *e.g.* by extraction. However, the above methods may sometimes be applicable even to true solutions, since mutual interaction of the pigment molecules in the particles of a suspension often does not influence the absorption appreciably, and a solvent may be found whose influence on the absorption of the pigment molecules is similar to that of the particle substance.

Correction for scattering in a suspension

In the above considerations, we assumed implicitly that it was possible to determine the "true" optical density of a suspension, α_{sus}, *i.e.* that the effect of scattering can be eliminated. Sometimes, the scattering can be minimized by adjusting the refractive index of the suspension medium. But if scattering cannot be removed a correction has to be applied to the measured optical density in order to find the true one. This correction may be carried out as follows. The absorption is measured by means of a conventional spectrophotometer in which the suspension and detector are not too far apart, for several suspensions of different concentration. At low concentrations, the scattering per unit volume is proportional to the number of scattering particles; the curve, representing the measured absorption as a function of the concentration, is a straight line through the origin. At high concentrations, however, part of the scattered light will be scattered again, and reach the detector; the curve will thus bend away from the y-axis. For suspensions of a concentration lying in the straight line region, the measured optical density $\alpha\ (\lambda)$ is approximately:

$$\alpha\ (\lambda) = \alpha_{\text{sus}}\ (\lambda) + s\ (\lambda) \tag{22}$$

where $s(\lambda)$ is the scattering, and $\alpha_{\text{sus}}\ (\lambda)$ the optical density if no scattering occurs; $\alpha_{\text{sus}}\ (\lambda)$ is the quantity needed for our calculations. If it is possible to bleach or remove the pigments by strong light or by chemicals, without changing the scattering of the particles, the apparent optical density of such a bleached suspension will be equal to $s\ (\lambda)$, and $\alpha_{\text{sus}}\ (\lambda)$ can be calculated by means of eq. (22). For spheres showing scattering due to change in refractive index at the surface only, computation of the scattering is, in principle, possible by means of equations derived by MIE[4], but the actual calculation will probably be time-consuming, even with the help of tables[5].

For many pigments, the true absorption drops to zero beyond a strong absorption band. Then the measured absorption of a suspension in the region beyond this band is caused by scattering. The scattering at shorter wavelengths may be estimated by extrapolation.

None of the suggested methods for the determination is completely satisfactory. The best method would be to measure the scattering of the suspension directly and to correct by calculation the measured scattering for the absorption in the suspension.

Applications to photosynthesizing cells

The above procedures were developed for and have been applied to the determination of the light absorptions of the individual absorbing structures in suspensions of photosynthesizing cells. Some of the results have been reported[1]. The procedure may be outlined using data obtained by EMERSON AND LEWIS[6]. The measured absorption spectrum of an extract of *Chlorella* was compared with that of a suspension of *Chlorella* containing the same quantity of pigment. The extinction of the suspension was partly due to scattering, as was indicated by an appreciable extinction at 720 mμ, where the pigments possess no absorption. The relationship between absorption and concentration for suspension of *Chlorella* was found by us to be linear in the region of concentration used by EMERSON AND LEWIS.

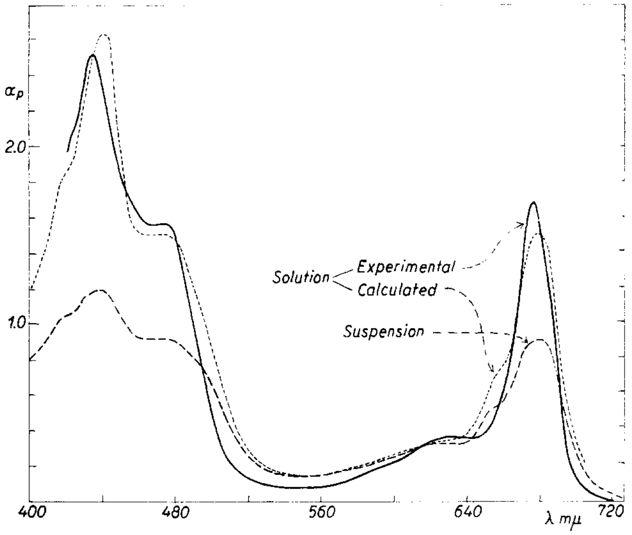

Fig. 3. Absorption spectra of a *Chlorella* suspension, of a quantitative methanol extract, and of the calculated spectrum of a solution of the cell pigments. The experimental spectra are, with one modification (see text), according to EMERSON AND LEWIS[6].

We applied equation (22) and assumed that the part of the extinction caused by scattering $s(\lambda)$, was constant and equal to the extinction at 720 mμ, where no true absorption occurred. If allowances were then made for the scattering and for the shifts of the absorption maxima which occur upon extraction, the absorption spectrum of the suspension appeared lower than that of the methanol solution (Fig. 3).

The experimental curves are, except for the scattering correction for the suspension, the same as those of EMERSON AND LEWIS[6]. We assumed that the absorbing structures in the *Chlorella* suspensions were homogeneously colored identical spheres. The choice of one parameter is left: the absorption of a sphere or its Q_A value at a certain wavelength. If this parameter is chosen, the absorption spectrum of a solution containing dispersed pigment molecules without change in intrinsic absorption can be

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calculated with the aid of Fig. 1. A spectrum calculated for one such a parameter value is shown in Fig. 3. The good correspondence with the experimental spectrum suggests that, except for band shifts, the discrepancy between the spectra of *Chlorella* and methanol extract is mainly caused by the geometrical effect of flattening, and not by a pronounced change in intrinsic absorption. The ordinate a refers to the calculated spectrum and is the optical density (ln I_0/I) through the center of the sphere. The corresponding mean percentage absorption of the sphere A_p can be read from Fig. 1. It is 60% at 680 mμ. The correctness of this method for determination of absorption is indicated by the fact that about the same absorption at 680 mμ was determined with a method based upon reabsorption of fluorescence light (DUYSENS AND HUISKAMP, *cf.*[1]). Similar procedures were successfully applied to suspensions of other species of unicellular photosynthesizing organisms. A satisfactory analysis of the absorption spectrum of photosynthesizing cells was thus made possible.

When this was written we heard (RABINOWITCH, personal communication) that LATIMER in RABINOWITCH's laboratory found a selective scattering in the absorption bands of *Chlorella*. This would mean that the flattening and thus the absorption of *Chlorella* must be greater than we estimated. Since LATIMER measured only the scattering perpendicular to the measuring beam it was not possible to estimate how much our result should be corrected. The correction may be small.

The application of the method to biological systems, other than photosynthetic organisms, seems promising. For example, it may be possible to determine, without removal from the cell, the near-ultraviolet optical densities of nuclei or other particles containing substances whose absorption spectrum extends to longer wavelengths than does that of the other cell constituents.

Advantages of the above method of calculating the true absorption curves are:

1. The method is applicable not only to suspensions of large particles but presumably also to suspensions of particles below microscopic visibility.
2. The apparatus required is relatively simple: the only major instrument needed is a conventional spectrophotometer.
3. For measurement of the absorption spectrum of the suspension, the particles need not be removed from the structure in which they are imbedded (provided this structure does not absorb in the spectral region under investigation).

If the absorption, $a_p(\lambda)$, of a particle has been determined, the size of the particle may be calculated by means of eq. (10), when $\gamma(\lambda)$, the specific absorption of the substance of the particle, is known. On the other hand, if the size of the particle is known, $\gamma(\lambda)$ may be calculated from $a_p(\lambda)$ by means of the same equation.

The above-mentioned method is of course not restricted to particles of biological origin, but can be applied to suspensions of absorbing particles in general, with the exception of such particles, *e.g.* metallic ones, in which the absorption is not localised in the individual absorbing molecules.

When comparing the absorption spectra of a suspension and corresponding solution, no conclusions concerning changes in absorption of individual molecules should be drawn without taking the "flattening" effect in account.

I am grateful to Dr. E. I. RABINOWITCH for discussion of the manuscript.

SUMMARY

It is proved that the absorption spectrum of a suspension of particles is flattened, as compared with that of a solution of the pigments contained in the particles. This flattening is due to the enhanced mutual shading of the pigment molecules in the suspension. A quantitative theory of this flattening is given. If the flattening is measured experimentally, the optical density of the individual particles can be computed, at least in principle (for spherical particles a nomograph is given to this purpose). This optical density may be used for the calculation of the "true" absorption spectrum of a pigment in suspension (which can be used, for example, for an improved analysis of a composite spectrum, in terms of overlapping absorption spectra of the several pigments present in the particle). Such an analysis is generally required when relative photochemical efficiencies of these pigments must be determined.

RÉSUMÉ

Il a été prouvé que le spectre d'absorption d'une suspension de particules est plus bas que le spectre d'une solution des pigments présents dans les particules. L'effet est causé par l'ombre mutuelle des particules. Une théorie quantitative est dérivée. Si l'effet a été mesuré, la densité optique des particules individuelles peut être calculée, du moins en principe (pour des particules sphériques, des nomographes sont construits). La densité optique peut être utilisée pour le calcul du "vrai" spectre d'absorption d'un pigment en suspension (lequel peut être utilisé, par exemple, pour une analyse perfectionnée d'un spectre composé d'une solution). Une telle analyse est généralement requise, lorsque les activités photochimiques relatives de ces pigments doivent être déterminées.

ZUSAMMENFASSUNG

Es wurde gezeigt, dass das Absorptionsspektrum einer Suspension kleiner Teilchen "flacher" ist als das Spektrum einer Lösung der in den Teilchen anwesenden Farbstoffe. Die Abflachung wird verursacht durch die gegenseitige Beschattung der Teilchen.

Eine quantitative Theorie dieses Effekts ist gegeben worden. Wenn der Effekt gemessen ist, kann man — wenigstens im Prinzip — die optische Dichte der einzelnen Teilchen berechnen (für kugelförmige Teilchen sind für diesen Zweck Nomographen gegeben). Diese optische Dichte kann benutzt werden für die Berechnung des "wahren" Absorptionsspektrums einer Suspension. Dieses Spektrum kann nun analysiert werden als die Summe der Spektren der anwesenden Pigmente. Eine solche Analyse ist im allgemeinen notwendig wenn die relativen photochemischen Wirksamkeiten dieser Pigmente bestimmt werden sollen.

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Received June 21st, 1955