Reprinted from Applied Optics, Vol. 27, page 3954, October 1, 1988

Modeling the light attenuation and scattering by spherical phytoplanktonic cells: a retrieval of the bulk refractive index

Dariusz Stramski, Andre Morel, and Annick Bricaud

Universite Pierre et Marie Curie, Laboratoire de Physique et Chimie Marines, B.P.8., F06230 Villefranche-sur-Mer, France.

Received 30 March 1988.
0003-6935/88/193954-03$02.00/0.

This Letter is an extension of an earlier paper[1] (referred to hereafter as BM) that described a theoretical modeling of light attenuation and scattering by pigmented phytoplanktonic cells in the common case when reliable and independently assessed data on spectral refractive index are lacking. The present work conforms to the notation and symbols previously used.

In essence, the BM model was developed, first, to determine whether the spectral scattering properties of algal cells can be adequately explained and reproduced when the (experimental) spectral absorption and the actual size distribution of the cells are known (and used as input parameters in the computation); second, to test the applicability of the anomalous diffraction approximation of van de Hulst[2] for algal cells that can significantly deviate from the idealized case of perfectly homogeneous spheres; and, finally, to predict backscattering properties of these cells. Note that the phytoplanktonic cells are characterized by the complex index of refraction, $m = n - in'$, so that $|m - 1|$ remains very close to zero.[3]

In the BM model the wavelength-dependent imaginary part $n'(\lambda)$ of the refractive index is derived from the measured absorption spectrum by an iterative procedure. The theoretical absorption efficiency factor $\bar{Q}_a(\tilde{\rho}')$ [Eqs. (10),(2') in BM] is forced to equal the experimental value of that factor by allowing n' to vary, thus providing its correct value. The overbars indicate that the efficiency factors are computed for a mean cell of the actual polydispersed population. The spectral values of the real part $n(\lambda)$ are written as $n(\lambda) = 1 + \epsilon + \Delta n(\lambda)$. The wavelength independent quantity $1 + \epsilon$ is referred to as the central value because the real part n differs, in general, from $1 + \epsilon$ by Δn due to the effect of absorption. $\Delta n(\lambda)$ is linked to $n'(\lambda)$ through the anomalous dispersion (Ketteler-Helmholtz) theory and is computed after the n' spectrum has been decomposed into several distinct oscillators with appropriate resonance peaks and damping constants [Fig. 5 and Eqs. (11),(5),(6) in BM].

When using the anomalous dispersion theory, the choice of ϵ is crucial as diverse patterns can result in the modeled efficiency factors for attenuation $\bar{Q}_c$, scattering $\bar{Q}_b$ (and backscattering $\bar{Q}_{bb}$), according to the value assigned to ϵ (Fig. 6 in BM). In the BM model a first guess for ϵ is based on a graphic comparison of the experimental spectrum of the efficiency factor for attenuation $\bar{Q}_c$ with that of the so-called nonabsorbing equivalent particle, $\bar{Q}_c^{NAE}$, computed through the van de Hulst approximation. A plausible range for $\tilde{\rho}$ compatible with the range of experimental $\bar{Q}_c(\lambda)$ values can, in principle, be delimited; hence ϵ can be estimated. Recall that the hypothetical nonabsorbing equivalent population (NAE) has the same characteristics as the actual one except that $n' = 0$ and $n = 1 + \epsilon$ everywhere in the spectrum. It was then indicated that the ϵ value can be refined by trials and errors comparing the $\bar{Q}_c$ spectrum predicted from the van de Hulst approximation with the experimental one. Note that even if the measured attenuation spectrum is not, strictly speaking, an input parameter in the BM model, it is implicitly used as such, at least in a qualitative manner, when the first guessed value of ϵ is adopted.

If now, and conversely to the second aim of BM, we *a priori* rely on the van de Hulst approximation because the algal cells under consideration are essentially homogeneous and spherical, the situation is different from that envisaged in the BM model; refinements and simplification in the computational scheme become possible, namely,

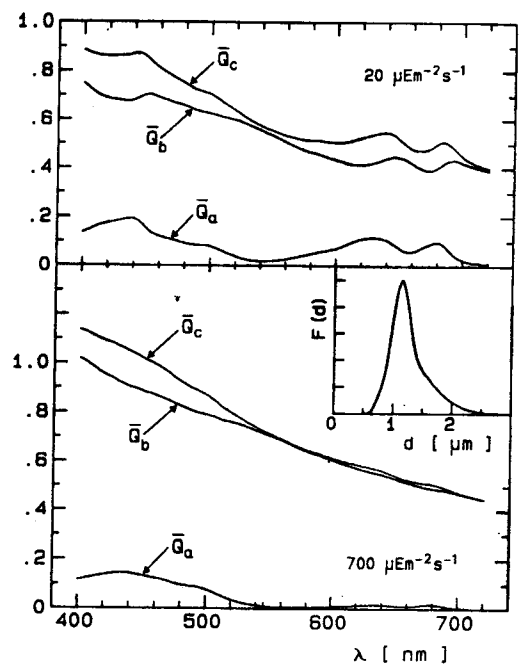

Fig. 1. Spectra of efficiency factors for attenuation $\bar{Q}_c$, scattering $\bar{Q}_b$, and absorption $\bar{Q}_a$ of the cyanobacterium *Synechocystis* deduced from the spectrophotometric measurements of absorption and attenuation and from the cell size distribution determined with the electronic particle counter combined with an epifluorescence technique for cells counting. In both panels, the growth irradiance for the cyanobacterium culture is indicated. The inset shows the mean size distribution of the *Synechocystis* population (in relative units). This distribution is practically insensitive to changes in growth irradiance.

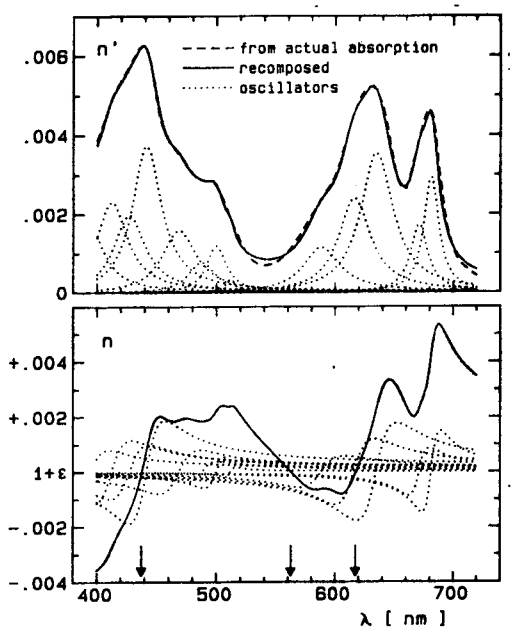

Fig. 2. Upper panel: the decomposition of the $n'(\lambda)$ spectrum into distinct oscillators and its recomposition for the *Synechocystis* culture grown under 20 $\mu E \cdot m^{-2} s^{-1}$. Lower panel: the spectral variations of the real part of the refractive index (solid line) as recomposed from the oscillators (dotted lines). The wavelengths λ_ϵ are indicated by arrows.

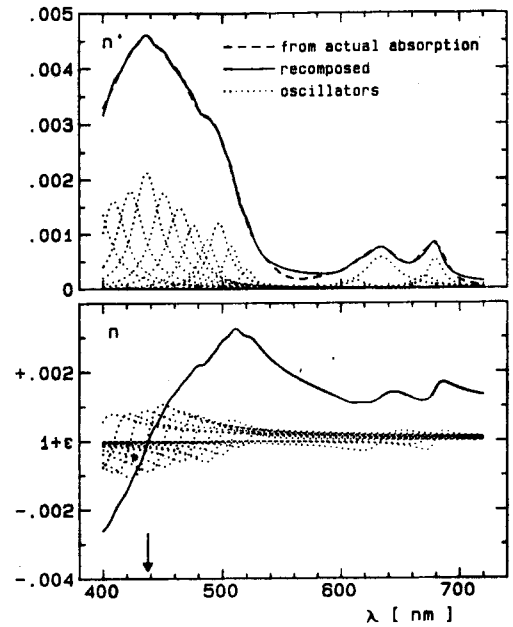

Fig. 3. As in Fig. 2 but for growth irradiance of 700 $\mu E\ m^{-2} s^{-1}$ (note change of ordinate scales from Fig. 2).

(1) when applying the anomalous dispersion theory the choice of ϵ, while retaining physical consistency, can be made on a mathematically well-defined basis;

(2) the $n(\lambda)$ spectrum can be obtained without appealing to the anomalous dispersion theory, i.e., without requiring the decomposition into oscillators.

The input parameters are now explicitly three: the absorption spectrum transformed into $\bar{Q}_a(\lambda)$; the attenuation spectrum transformed into $\bar{Q}_c(\lambda)$; and the size distribution function $F(d)$. The aim of this Letter is to present the modified computational scheme and to compare the results with those obtained through the BM model, when applied to an almost ideally spherically shaped cyanobacterium cell (*Synechocystis*, *Cyanophyceae*), with a mean diameter of $\sim 1.2\ \mu m$. Absorptive and scattering properties of this picoplanktonic species are strongly variable in response to growth irradiance making such a cell an interesting optical object.[4] The data that serve here as input parameters are shown in Fig. 1; the measuring methods are described elsewhere.[1,4]

As regards (1), recall that the comparison of $\bar{Q}_c^{NAE}$ and $\bar{Q}_c$ spectra (as in BM) is not fully justifiable because the experimental $\bar{Q}_c(\lambda)$ values are naturally affected by absorption *a priori* anywhere inside the entire (visible) spectrum. Therefore, a refined procedure for selecting the central value $1 + \epsilon$ can be imagined, which is formulated as follows. Figure 2 shows, for the cyanobacteria adapted to low growth irradiance (20 $\mu E\ m^{-2} s^{-1}$), the $n'(\lambda)$ spectrum derived from the actual absorption by iterative scheme (as in BM), its decomposition into oscillators and the spectral behavior of the recomposed $n(\lambda)$ around the unknown central value $1 + \epsilon$. In this case thirteen oscillators lead to almost perfect recomposition of the $n'(\lambda)$ spectrum. The real part of the refractive index displays appreciable variations with wavelength. The analogous results but for the cyanobacteria grown under high irradiance (700 $\mu E\ m^{-2} s^{-1}$) are shown in Fig. 3. The $n'(\lambda)$ absorption pattern, recomposed with fifteen oscillators, strongly differs from the preceding one. In general, a good reconstruction requires many trials and is difficult to achieve when the $n'(\lambda)$ spectrum is strongly structured with very low values at absorption minima. When looking at the recomposed $n(\lambda)$ spectra (lower panels and heretofore published results[1,3]) it appears that, within the spectral range considered, Δn can be zero at least once and in some cases at several wavelengths, denoted λ_ϵ. As expected, one of these wavelengths is generally found in the vicinity of the blue absorption peak around 440 nm (perhaps except when the absorption remains relatively high at the shorter wavelength side of the spectrum). At wavelengths λ_ϵ, where n is exactly $1 + \epsilon$, the $\bar{Q}_c(\lambda_\epsilon)$ can be used to infer ϵ by a simple method. Having fixed wavelength λ_ϵ, $\bar{Q}_c^{NAE}$ is generated as a function of $\bar{\rho}$ and in fact as a function of the single variable ϵ, since the size distribution is given. Recall that taking into account the polydispersion the $\bar{Q}_c^{NAE}(\bar{\rho})$ are calculated according to

$$\bar{Q}_c^{NAE}(\bar{\rho}) = \int_0^\infty Q_c(\rho) F(\rho) \rho^2 d\rho \left[\int_0^\infty F(\rho) \rho^2 d\rho \right]^{-1}, \qquad (1)$$

where $F(\rho)$ is obtained from the experimental size distribution by the replacement of d by ρ and $Q_c(\rho)$ from van de Hulst's formula assuming $\zeta = 0$:

$$Q_c(\rho) = 2 - (4/\rho) \sin\rho + (4/\rho^2)(1 - \cos\rho). \qquad (1')$$

Finally, the exact value of ϵ is indicated by such $\bar{Q}_c^{NAE}(\bar{\rho})$ that it equals $\bar{Q}_c(\lambda_\epsilon)$. $\bar{Q}_c^{NAE}$ is a nonmonotonic function of $\bar{\rho}$, more than one value of $\bar{\rho}$ can thus provide $\bar{Q}_c^{NAE}$ equal to $\bar{Q}_c(\lambda_\epsilon)$. To overcome this ambiguity a plausible domain for $\bar{\rho}$ is easily delimited, as has been explained previously.[1] Note, however, that the method for selecting ϵ is inefficient for large $\bar{\rho}$ where $\bar{Q}_c$ becomes nearly independent on $\bar{\rho}$. The central values of n, determined here for the low light and high light adapted cyanobacteria, are 1.053 (±0.0019) and 1,0588, respectively. The former value is an average based on three wavelengths λ_ϵ, i.e., 437.5, 562.5, and 617.5 nm. As seen in

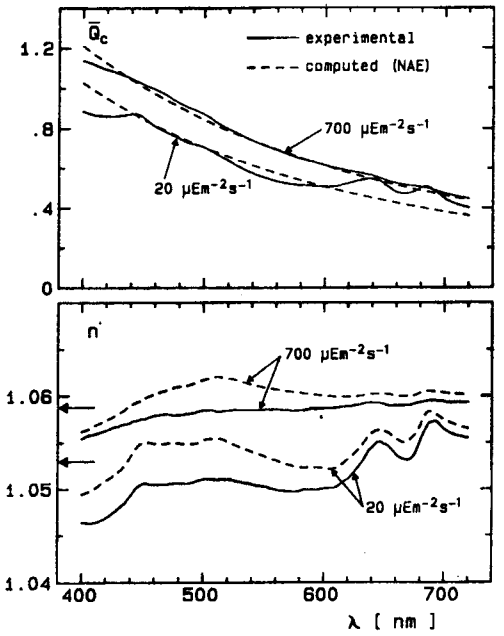

Fig. 4. Upper panel: the spectrum of the experimental efficiency factor for attenuation compared with that representative of an NAE cell computed for the determined central values $1 + \epsilon$. Lower panel: the real part of the refractive index obtained from the iterative scheme (solid lines) compared with that estimated from the decomposition into oscillators (dashed lines, the selected values of $1 + \epsilon$ are indicated by horizontal arrows). In both panels, the growth irradiance for the *Synechocystis* culture is indicated.

Fig. 4 (upper panel), the experimental spectra of $\bar{Q}_c$ are in excellent compatibility with $\bar{Q}_c^{NAE}$ spectra computed with the above values of $1 + \epsilon$. The fact that $1 + \epsilon$ is determined from and depends on the accuracy of the attenuation measurement at a single wavelength could be seen as a disadvantage of the proposed refinement. This can, at least partly, be overcomed by using a few discrete wavelengths around λ_ϵ and averaging the estimates of ϵ or using more than a single λ_ϵ if they exist. The important advantage, however, lies in that this procedure is mathematically well defined and eliminates guess intervention.

As regards (2), if again we rely on the van de Hulst approximation having available the experimental values of $\bar{Q}_a(\lambda)$ and $\bar{Q}_c(\lambda)$, the spectrum of the real part of the refractive index can be directly computed without appealing to the anomalous dispersion theory. Given that the imaginary $n'(\lambda)$ values have been derived (as in BM), the computational scheme allowing $n(\lambda)$ to be obtained is as follows. At each wavelength separately (2.5-nm interval), the theoretical value of $\bar{Q}_c(\bar{\rho})$ is iteratively forced to equal the experimental $\bar{Q}_c(\lambda)$ value. The varying parameter in this procedure is the real part of the refractive index n. To avoid possible multiple solutions, n is incremented within a plausible domain, and the iteration is pursued until the criterion $|[\bar{Q}_c(\bar{\rho}) - \bar{Q}_c(\lambda)]/\bar{Q}_c(\lambda)| < 10^{-3}$ is satisfied. Analogously to Eq. (1) the formula for $\bar{Q}_c(\bar{\rho})$ allowing for the effect of polydispersion is

$$\bar{Q}_c(\bar{\rho}) = \int_0^\infty Q_c(\rho)F(\rho)\rho^2 d\rho \left[\int_0^\infty F(\rho)\rho^2 d\rho\right]^{-1}, \quad (2)$$

where $Q_c(\rho)$ is computed from

$$Q_c(\rho) = 2 - 4\exp(-\rho\tan\zeta)[(\cos\zeta/\rho)\sin(\rho - \zeta) + (\cos\zeta/\rho)^2\cos(\rho - 2\zeta)] + 4(\cos\zeta/\rho)^2\cos 2\zeta. \quad (2')$$

In Fig. 4 (lower panel) the $n(\lambda)$ spectra computed by this iterative scheme are compared with those obtained from the decomposition into oscillators. As seen, the method based on the decomposition leads to higher values of $n(\lambda)$. In practice, the differences do not appear to be so significant (<0.005) and become easily understandable in view of the fact that the accuracy of each approach is affected by different sets of factors, namely, $\bar{Q}_a(\lambda)$ followed by the decomposition and choice of ϵ in BM instead of $\bar{Q}_a(\lambda)$ and $\bar{Q}_c(\lambda)$ in the direct iterative method.

Of crucial importance in both approaches is the accuracy of size distribution function. A sensitivity test showed that the deviations from the mean size distribution of cyanobacterium population, as actually detected by day-to-day observation, could lead to variations in the $1 + \epsilon$ estimate to within ± 0.0025. The iterative method for $n(\lambda)$ should obviously be used with caution if errors in experimental $\bar{Q}_c(\lambda)$ are suspected, possibly leading in extreme cases to unsuccessful convergence.

Summarizing, we conclude that both computational schemes, BM (including the refined procedure for selecting ϵ) and the iterative one, for determining $n(\lambda)$ are consistent to within experimental errors affecting the input data, provided that the van de Hulst approximation is applicable. The iteration method appears to be faster and more convenient for practical applications, whereas the tedious decomposition into oscillators provides a physical understanding of the spectral behavior of n. The present computational schemes are especially useful when studying the small sized picoplanktonic species having spherical shape and weakly pronounced internal structures.

Dariusz Stramski is on leave from the Polish Academy of Sciences, Institute of Oceanology. This work was done during a visit to the Laboratoire de Physique et Chimie Marines in Villefranche-sur-Mer, supported by Unesco grant SC/RP/236074.7.

References

1. A. Bricaud and A. Morel, "Light Attenuation and Scattering by Phytoplanktonic Cells: A Theoretical Modeling," Appl. Opt. 25, 571 (1986).
2. H. C. van de Hulst, *Light Scattering by Small Particles* (Wiley, New York, 1957).
3. A. Morel and A. Bricaud, "Inherent Optical Properties of Algal Cells Including Picoplankton: Theoretical and Experimental Results," Can. Bull. Fish. Aquat. Sci. 214, 521 (1986).
4. D. Stramski and A. Morel, "Optical Properties of Photosynthetic Picoplankton in Different Physiological States as Affected by Growth Irradiance" (in preparation).