RECONCILIATION OF THEORY AND EXPERIMENT ON 90° SELECTIVE SCATTERING SPECTRA AS A MEASURE OF INTACT OR BROKEN GRANAL OR AGRANAL CHLOROPLASTS

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1. Introduction

Early studies on 90° selective light scattering over 400–750 nm were made on the green alga *Chlorella pyrenoidosa* and the marine diatom *Navicula minima* [1]. These indicated peak values located in the 510–515 nm and 690–700 nm regions. *Chlorella* contains appressed chloroplast membranes but without the distinctive grana structures of higher plants. More recently, the selective scattering spectra at 90° of non-intact spinach chloroplasts were studied and scattering peaks about 518 nm and 690 nm were shown [2]. Light-induced slow 90° scattering changes in the 520 nm region were the principal purpose of these investigations and these were shown to be related to light-induced grana shrinkage by a corresponding theoretical analysis [3], and also to changes in the 650–750 nm region.

The experimental spectrum of 90° scattered light in the 650–750 nm region was later measured [4], for chloroplasts containing different degrees of grana, with the outer envelopes either intact or broken. They found that the scattering spectrum for intact granal chloroplasts had a peak at 703 nm, and non-intact chloroplasts gave a peak at 690 nm. They also calculated a theoretical 90° scattering spectrum in the red region by modelling the chloroplast as a homogeneous pigmented sphere by use of the van de Hulst approximation of Mie theory. This gave the total amount of light scattered by the sphere. Good agreement between the theoretical and experimental spectra was obtained for broken chloroplasts at 690 nm. However, for intact chloroplasts the peak of the theoretical curve also remained at 690 nm, instead of shifting as did the experimental peak to 703 nm. This could not be reconciled [4] by making reasonable changes to the particle parameters. They explained the frequency shift between intact and broken chloroplasts as being due to the decrease in chloroplast refractive index caused by dilution with the surrounding medium when the outer envelope was broken. However, the van de Hulst approximation does not reproduce this shift when such a difference in refractive index is taken into account.

In this letter it is shown that 90° light scattering is more sensitive to particle size, refractive index and incident light wavelength, than is total scattering, this latter being the quantity given by the van de Hulst approximation. It is necessary to use the full Mie theory to show that the theoretical scattering spectra reproduce the frequency shift given by the experimental 90° scattering spectra.

2. Comparison of total and 90° light scattering by chloroplasts

The van de Hulst approximation of Mie theory [5–8] allows the total amount of light scattered from a homogenous pigmented sphere to be calculated under the restrictions that the particle is large compared to the wavelength of light, and that the refractive index of the particle is close to that of the surrounding medium.

The advantage of this approximation over Mie theory is that it can be used to calculate the total amount of scattering from large particles, whereas the use of Mie theory becomes quite laborious. However, only Mie theory gives the light intensity scattered at 90°; the experimental quantity measured in [4].

The efficiency factor for the total amount of light scattered by a sphere is given by the van de Hulst approximation [5]:

$$Q_{sca1} = Q_{ext} - Q_{abs} \quad (1)$$

where

$$Q_{ext} = 2 - 4\exp(-\rho \tan\beta)(\cos\beta/\rho)\sin(\rho - \beta) - 4\exp(-\rho\tan\beta)[(\cos\beta)^2/\rho^2]\cos(\rho - 2\beta) + 4(\cos\beta/\rho)^2\cos 2\beta \quad (2)$$

and

$$Q_{abs} = 1 + \frac{\exp(-2\rho\tan\beta)}{\rho\tan\beta} + \frac{\exp(-2\rho\tan\beta) - 1}{2\rho^2\tan^2\beta} \quad (3)$$

$$\rho = \frac{4\pi a}{\lambda}(n-1) \quad (4)$$

$$\tan\beta = nk/(n-1) \quad (5)$$

where a is the particle radius and λ is the wavelength of light in the surrounding medium. The complex refractive index of the particle is defined by:

$$m = n(1 - ik) \quad (6)$$

Mie theory [5] gives the scattering efficiency as:

$$Q_{sca2} = 2/\alpha^2 \sum_{\ell=1}^{\infty} (2\ell + 1)\left(|a_\ell|^2 + |b_\ell|^2\right) \quad (7)$$

where a_ℓ and b_ℓ are functions of the size and refractive index of the particle and α is the size parameter:

$$\alpha = 2\pi a/\lambda \quad (8)$$

The intensity of light scattered at a particular angle is given by:

$$I_\theta = \frac{\lambda^2}{8\pi a^2}(i_1 + i_2) \quad (9)$$

where i_1 and i_2 are intensity functions dependent on the particle size, refractive index, the angle of scattering, and the polarization of the incident light.

Using both the van de Hulst approximation, eq. (1) and the full Mie theory, eq. (7), the total amount of scattered light has been calculated for a range of incident light wavelengths. The ratio of light scattered at 90° to the transmission at 0° I_{90}/I_0, has also been calculated using eq. (9) for a refractive index typical of chloroplasts, and for a range of particle radii. The results are plotted in fig.1. It can be seen that for a particle of 100 nm radius, I_{90} is insensitive to wavelength, and that it is approximated in shape reasonably well by the total scattering; given by either the van de Hulst approximation, Q_{sca1}, or by Mie theory Q_{sca2}. However, for a particle of 1000 nm radius, I_{90} varies much more with wavelength, and is poorly

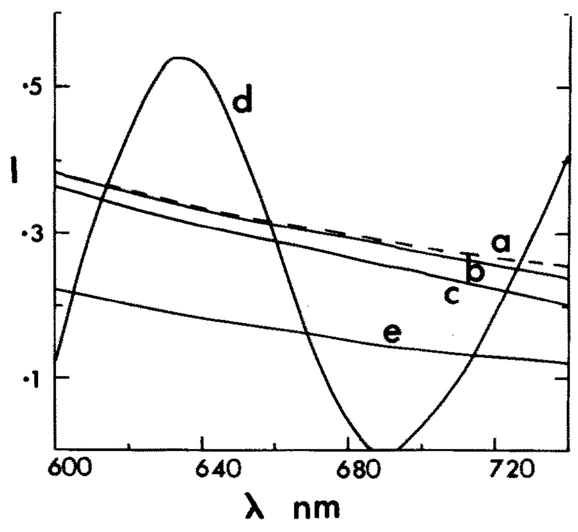

Fig.1. Variation of normalized total scattering intensity and 90° scattering intensity with wavelength. Index of refraction relative to medium, m = 1.01. Selective dispersion is not considered for these particles. (a) Q_{sca1}, a = 100 nm. (b) Q_{sca2}, a = 100 nm. (c) I_{90}/I_0, a = 100 nm. (d) I_{90}/I_0, a = 1000 nm. (e) Q_{sca2}, a = 1000 nm.

approximated by Q_{sca2}, the total scattering for the same particle. Since chloroplasts have the dimensions of ~5000 nm, this shows that Q_{sca2} will give inaccurate results in calculating the 90° scattering spectra of intact chloroplasts, and that eq. (9) for Mie theory giving the 90° scattering should be used.

3. Calculation of 90° scattering spectra using Mie theory

In order to calculate the 90° scattering from a suspension of chloroplasts, we model the chloroplasts as a homogeneous pigmented sphere and use eq. (9). The functions i_1 and i_2 depend on the complex refractive index of the sphere; the variation of this quantity within the absorption band can be modelled using the theory of selective dispersion [9], which gives the refractive index:

$$m = \left(1 + C_0 + \frac{C_1}{1 - (\omega/\omega_0)^2 - (i\gamma\omega/\omega_0^2)}\right)^{1/2} \quad (10)$$

where ω_0 is the frequency of the absorption peak; γ is the half width of the absorption band, C_0 is a constant determined by the index of refraction far from the absorbing band, and C_1 is a constant determined by the relative strength of the absorbing band.

To calculate the scattering spectrum for intact granal chloroplasts, we use in angular measure:

$$\omega_0 = 27.802 \times 10^{14}\ s^{-1}$$

$$\gamma = 1.583 \times 10^{14}\ s^{-1}$$

and from the absorption spectra [4] we find C_0 = 0.85 and C_1 = 0.0022. Using a particle radius of 2000 nm, which is typical of chloroplast size, the scattering spectrum was calculated for the angles 90° ± 3°, corresponding to the experimental situation in [4]. This is compared with the theoretical broken chloroplast spectrum in fig.2. The use of full Mie theory then gives agreement between experiment [4] and theory whereas the van de Hulst approximation is not appropriate.

When the chloroplast outer envelope is broken, it seems likely, as suggested [4], that the refractive index

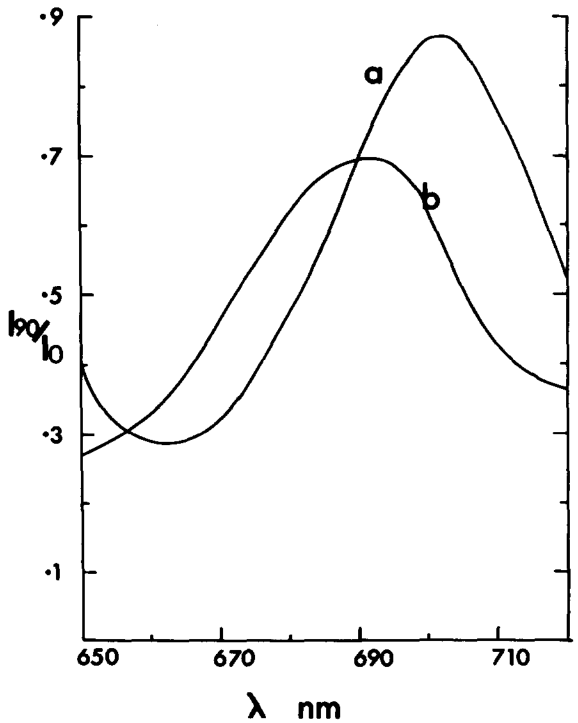

Fig.2. Theoretical 90° scattering spectra (normalized intensity). (a) I_{90}/I_0, a = 2000 nm, m = 1.013. (b) I_{90}/I_0, a = 2000 nm, m = 1.003. These spectra relate to chlorophyll-pigmented particles with selective dispersion present in both (a) and (b) (see text and eq. (10)).

of the chloroplast will decrease due to dilution by the outside medium. It is shown in fig.2 that when the particle refractive index is decreased by 1% the scattering peak moves to 690 nm, in line with the experimental peak. This provides a theoretical explanation for the experimental shift in the scattering peaks between intact and broken chloroplasts.

Class B agranal chloroplasts have a scattering peak at 682 nm. The above analysis shows that 90° scattering is very sensitive to particle size, and that as noted [4], this blue shift is probably due to Rayleigh scattering from the large number of small chloroplast fragments.

4. Conclusion

It has been shown that the 90° scattering from particles of the size and refractive index of chloroplasts, is a much more sensitive function of incident wavelength, particle size and refractive index, than is the total intensity of light scattered from the particle. As a result of this the full Mie theory should be used in comparing theoretical and experimental 90° scattering spectra.

It is also the purpose of this letter to alert biologists, intent on using 90° light scattering spectra in studies on particulates the size of the light wavelength order or greater, that the full Mie scattering theory should be used [5]. Where such studies include absorption bands when selective dispersion also occur, then the Mie function serves to modulate the selective dispersion effect.

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