TOTAL CROSS SECTION FOR OPTICAL SCATTERING BY SPHERICAL CELLS IN SUSPENSION

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INTRODUCTION

When light passes through a solution or a suspension, the attenuation of its magnitude is caused by scattering as well as by absorption. In macromolecular chemistry, light scattering has been studied for the purpose of evaluation of molecular weight, while in microbiology it seems to afford a means for the estimation of variations in cell population. Theories have well been developed by RAYLEIGH and GANS (1) for the cases in which the diameter of scatterers is smaller than the wave length of light as in macromolecular chemistry. When the diameter of scatterers is larger, as often met in microbial cell suspensions, than the wave length of the rays used, theoretical analysis of scattering phenomena has to be performed in accordance with the MIE's rigorous expression (2). Owing to the lack of an analytically expressed formula, a number of experimental studies have been conducted in the field of microbiology so far without theoretical basis.

In the present paper, it will experimentally be shown that a simplified formula originally given by HART and MONTROLL (3) holds well for the total cross section of optical scattering in the suspension of spherical cells with the diameter of 10^{-4} cm in order. The expressions is much more compact in form and will make as illustrated in the following papers a convenient working basis for the analysis of scattering spectra in cell suspensions.

THEORETICAL

For the sake of simplicity, unicellular organisms of spherical (or quasi-spherical) form will be taken. Disregarding, for the present, complicated internal structures, we may assume the cells as optically homogeneous spheres having geometrical radius a and effective refractive index n_1 in common. This single phase model allows us to apply the same mode of analysis as in non-biological systems. As is well known, numerical computations involved in MIE's scheme is formidably time consuming for the related cases in which the ratio of diameter to wave length amounts to several tens in order. Mathematical tables presented thus far are, moreover, incomplete

for biological uses due to the fact that relative index of refraction varies in the narrow range close to unity.

An alternative formula was presented by HART and MONTROLL for the total cross section of soft scatterers as follows:

$$\sigma_s/\pi a^2 = \{\pi x^2(m-1)^2/2m\} \{ (m^4 + 6m^2 + 1)A_1 - 2(m^2 + 1)x^{-2}A_2 + x^{-4}A_3 \} , \quad (1)$$

where

$$A_j = I_j(x[m+1]) + I_j(x[m-1]) ,$$

with

$$I_1(2x) = (1/2\pi) - (1/8\pi) \{ J_{1/2}^2(2x) + J_{3/2}^2(2x) \} ,$$

$$I_2(2x) = (1/\pi) \{ (\sin 4x/2x) - (\sin^2 2x/4x^2) - 1 + \varphi(4x) \} ,$$

$$I_3(2x) = (1/\pi) \{ 2x^2 + x \sin 4x - (5/2) \sin^2 2x + \varphi(4x) \} ,$$

and

$$\varphi(z) = \int_0^z (1 - \cos y) dy/y .$$

On the left hand side of the equation (1), σ_s is optical cross section for scattering per cell. If multiple scattering does not occur, σ_s is related to turbidity (τ) as follows:

$$\sigma_s = \tau/N ,$$

where τ is experimentally given by

$$I = I_0 e^{-\tau l} ,$$

I_0 : Intensity of incident light

I : Intensity of transmitted light

l : Length of optical path in cm.

On the right hand side of the equation (1), relative index of refraction (m) is defined by

$$m = n_1/n_0 ,$$

n_1 : Index of refraction of scatterer

n_0 : Index of refraction of suspending medium

and ratio parameter x is defined by

$$x = 2\pi a/\lambda_0 ,$$

where λ_0 is the wave length of incident light in the suspending medium. Fortunately, relative index of refraction of biological materials is nearly 1 owing to the high content of water in cells. Equation (1) takes, accordingly, a simpler form as follows:

$$a_s = 2 - 4\rho^{-1} \sin \rho + 4\rho^{-2}(1 - \cos \rho),$$

$$\rho = 2x(m-1). \quad (2)$$

Equation (2) is identical with the expression derived independently by VAN DE HULST (4) for the range $x \gg 1$.

To check the theoretical errors inherent in this approximate formula, the curve of σ_s computed by equation (2) is compared with the values of σ_s given in accordance with the MIE's rigorous expression for $m=1.05$ and $m=1.10$ by using PANGONIS-HELLER-JACOBSON'S numerical tables (5). The agreement is fairly satisfactory for biological uses as shown in Fig. 1.

The validity of equation (2) may break down for the range $\rho \lesssim 1$. Since, as is well known, RAYLEIGH-GAN'S expression represented as follows holds well in this range, equation (2) used in combination with equation (3) will cover the whole range of related problems.

Fig. 1. Approximate curves of $\sigma_s/\pi a^2$ in accordance with equation (2). Open circles and Crossed bars represent exact values given by the numerical tables (5).

$$\sigma_s/\pi a^2 = (m-1)^2 \phi(x),$$

$$\phi = (5/2) + 2x^2 - (\sin 4x/4x) - (7/16x^2)(1 - \cos 4x)$$

$$+ \{(1/2x^2) - 2\} \{0.577 + \log 4x - C_i(4x)\},$$

$$C_i(x) = - \int_x^\infty (\cos u/u) du. \quad (3)$$

For rough estimation of σ_s in the range $0.2 \lesssim \rho \lesssim 1$, equation (3) may be replaced by the following simpler expression.

$$\sigma_s/\pi a^2 = (m-1)^2 2x^2 = (1/2)\rho^2. \quad (4)$$

APPARATUS

In order to obtain experimentally the value of σ_s , the absolute value of turbidity (τ) must be found for the suspensions, the population of which is so small that multiple scattering has no effect on it. A correct value of τ can be secured either through the transmission measurement under a narrow beam condition or by the integral measurement of scattered light. An experimental apparatus constructed based on the former principle without the use of lens systems. The collimator part consisted of four identical narrow circular slits as shown in Fig. 2. The size of the slit was determined so as to give a correct value of total scattering with errors less than 5%. If the upper limit of cell size is assumed 10^{-3} cm in diameter, the al-

Fig. 2. Experimental apparatus for transmission measurement under narrow beam condition.

<i>W</i> : tungsten lamp	<i>S</i> : slit system (2×100 mm)
<i>F</i> : neutral filter (×1/1, ×1/70) or interference filter	<i>C</i> : sample cell (10×10×50 mm)
<i>L</i> : collimator lens (as part of light source)	<i>PM</i> : photomultiplier (IP-28)
	<i>G</i> : galvanometer (10 ⁻⁹ A/mm)
	<i>M</i> : galvanometer shunt

lowable maximal diameter of circular slit was estimated to be 2 mm for the dimensions of apparatus adopted. The larger is the size of a scatterer, the more light is scattered in the forward direction. The estimation was, therefore, roughly made for the case of largest scatterer under consideration by using the Fraunhofer diffraction formula for the angular distribution of scattered light at small angles. By this apparatus the measurement of extinction was possible for suspensions with τ from 0.1 to 5 in order. This range in τ covers half a decade in logarithmic population, e.g. 10⁴~10⁸ cc in the case of *Saccharomyces cerevisiae*.

EXPERIMENTAL

(1) Scattering Cross Section

In order to eliminate the effect of multiple scattering, extrapolation to zero population was made by means of the following empirical expression:

$$\tau_n = N/(\alpha + \beta N), \quad (5)$$

τ_n : Turbidity at population N

N : Population density of suspension

or

$$N/\tau_n = \alpha + \beta N.$$

These equations are identical in form with those used for the turbidity of macromolecular solution. As equation (5) takes the form $\tau_n = N/\alpha$ when N tends to zero, we get

$$\sigma_s = 1/\alpha.$$

The value of α will, therefore, be obtained as an intercept on y-axis of $(N/\tau_n) - N$ diagram.

$\tau_{10} (\equiv \tau_n/2.30)$ observed for suspensions of washed cells was plotted

Fig. 3. Optical extinction against population density (*Saccharomyces cerevisiae*).

Fig. 4. Optical extinction against population density (*Rhodotorula glutinis*).

Fig. 5. Optical extinction against population density (*Micrococcus luteus*).

Fig. 6. N/τ_{10} vs. N (*Saccharomyces cerevisiae*).

Fig. 7. N/τ_{10} vs. N (*Rhodotorula glutinis*).

Fig. 8. N/τ_{10} vs. N (*Micrococcus luteus*).

against N as shown in Fig. 3, Fig. 4 and Fig. 5. Diagrams of (N/τ_{10}) versus N were obtained therefrom as drawn in Fig. 6, Fig. 7 and Fig. 8, giving

$\sigma_s = 7.6 \times 10^{-7} \text{ cm}^2$, $3.3 \times 10^{-7} \text{ cm}^2$ and $4.2 \times 10^{-9} \text{ cm}^2$ for *Saccharomyces*, *Rhodotorula* and *Micrococcus*, respectively.

(2) Intra-cellular Refractive Index

Two methods were adopted to obtain the value of cell index of refractions (n_1). Neither of these used any functional form related to the scattering equations described above.

(a) Microscopic Method—Individual cells were observed under a phase-microscope in the medium of varying index (n_0) of refraction. Phase contrast disappears when n_0 takes the same value as n_1 . In order to vary the value of n_0 , serum albumin (6) was added to a basal medium. Any substance with high solubility will do, provided that this molecular weight is sufficiently large to maintain the osmotic balance of the cells. By using a sodium-lamp, 1.40, 1.39 and 1.38 were found as values of n_1 for *Saccharomyces cerevisiae*, *Rhodotorula glutinis* and *Micrococcus luteus*, respectively.

(b) Minimum Scattering Method—Minimal turbidity points were found for the cell suspensions in the series of media with varying index of refraction (n_0). As is shown in Fig. 9, n_1 of *Saccharomyces cerevisiae* was found 1.41 at the wave length of 1μ . Since much larger quantity of adding substances was necessary than in the former case, sucrose was used. The molecular weight of sucrose is not so large as to prevent osmotic volume change in cells, but, since the effect of volume contraction counteracts the effect of increase in refractive index of cells in this case as will be shown in the succeeding paper, the obtained value can be considered correct to a first approximation.

Fig. 9. Optical extinction of cell suspensions at 1μ with varying n_0 (*Saccharomyces cerevisiae*).

Fig. 10. Observed scattering cross sections compared with the theoretical curve.

(3) Comparison with Theory and Discussion

Taking 1.40 as an average values of refractive index for the cells used, observed values of scattering cross section are well fitted as shown in Fig. 10 by a theoretical curve brawn by means of equation (2) for $\rho > 1$ and equation (3) for $\rho < 1$. Cell populations and average cell diameters necessary

for the computation were obtained, thereby, in a similar way as shown in a previous paper (7).

In order to obtain better agreements, many factors, the effects of which were disregarded in the present paper, must be taken into consideration: e.g., departure of cells from spherical in shape, distribution of cell diameters, wave length dispersion of refractive indices, optical heterogeneity of cell content, etc. The approximate representation shown in this paper will, however, prove fairly useful in its simple form for many biological purposes.

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SUMMARY

(1) Absolute values of turbidity in cell suspensions were measured under the narrow beam condition, giving, thereby, correct values of scattering cross section per cell.

(2) By using zero method, average indices of refraction were obtained for *Saccharomyces cerevisiae*, *Rhodotorula glutinis* and *Micrococcus luteus*.

(3) If cells are assumed as homogeneous spheres, the cross section could be computed by RAYLEIGH-GANS' and HART-MONTROLL's expressions. The computed values agreed well with the observed ones for the specimen used.

(4) The equations mentioned above will make, accordingly, a convenient theoretical basis for further studies on scattering of light in cell suspensions as will be shown in succeeding papers.

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