THE SMALL ANGLE LIGHT SCATTERING OF BIOLOGICAL CELLS

THEORETICAL CONSIDERATIONS

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ABSTRACT The light scattered by living biological cells (assumed homogeneous spheres with a relative refractive index, $m = 1.05$) at small angles has been calculated by the Hodkinson approximation and the more rigorous Mie theory. Both methods predict that relative volume distributions may be estimated from low angle scattering measurements on each cell in a population. Under conditions of short wavelength illumination or strong absorption, absolute volume information may also be obtained.

INTRODUCTION

Within recent years several workers have been concerned with the scattering of light by biological cells, usually in an effort to obtain size and shape information. Koch (1–3) and Wyatt (4) have applied the Rayleigh-Gans (5, 6) method to the analysis of bacterial scattering. Latimer and his coworkers (7–9) have done a considerable amount of theoretical and experimental work on cells and other particles of similar size.

Flow photometers for the rapid measurement of fluorescence (10) and small angle light scattering (11) of mammalian cells have been developed at this laboratory. In the scattering photometer, each cell produces a pulse of scattered light as it crosses a beam of laser light; the angular position of the detector is fixed. It is necessary, therefore, to determine the relationship between the scattered light intensity, particle size, and scattering angle. Results of this theoretical analysis and the suggested experimental approaches are reported here.

CELL MODEL AND SCATTERING PROCESS

In scattering theory, it is usual to refer to particle size in terms of a dimensionless quantity, $\alpha = \pi d/\lambda$, the ratio of the particle circumference to the wavelength of the incident light. For $\lambda = 500$ nm, many mammalian cells are in the range $50 \leq \alpha \leq 100$.

For particles this large, scattering calculations based on the rigorous Mie theory (12) or an appropriate large particle approximation must be used. The scattering particle is usually assumed to be a homogeneous sphere of uniform refractive index m_1 immersed in a medium of refractive index m_2 ; the relative refractive index $m = m_1/m_2$.

For particles larger than a few wavelengths ($\alpha > 10$), the scattering may be considered as composed of contributions from Fraunhofer diffraction, refraction (transmission), and external reflection (13, 14). Although this approach is not exact, it does give some insight into the scattering process.

The diffraction pattern from a sphere of cell size consists of a strong central maximum (containing over 80% of the diffracted light) surrounded by a series of secondary maxima and minima. Diffraction, independent of m, is a strong function of α with the angular position of the edge of the central maximum given by $\alpha \sin \theta_{\min} = 3.83$. Thus, most of the light diffraction by a 10 μ diameter sphere illuminated with visible light ($\lambda = 500$ nm) is within 4° of the forward direction. External reflection and refraction contributions are functions of m. For cells, $m \approx 1.05$, the reflection contribution is negligible for this α range if the scattering angle, θ, is less than 25° and may be ignored as a scattering mechanism for the cases presented here.

The refraction contribution is a strong function of m as $m \to 1$. One should expect the interior structure of the particle to determine the nature of the transmitted light for cells with $m = 1.05$. We estimate, using the Hodkinson approximation (14), that for $30 \leq \alpha \leq 120$, the refraction contribution ranges from 29 to 7%, respectively, of the diffraction contribution at a scattering angle of 0.5°. Thus, a scattering measurement made at very small angles within the diffraction-dominated region should yield information on α (the gross size of the cell) and be relatively insensitive to other cellular features.

METHODS

We have employed three approaches: (*a*) simple diffraction, (*b*), the approximation of Hodkinson, and (*c*) the exact Mie theory for spheres. We will be mainly concerned with the Mie results in this paper. The diffraction and Hodkinson calculations were done on an IBM 7094 with locally generated codes. The Mie theory codes DAMIE and DBMIE developed by Dave (15) were run on a CDC 6600. Although there are some internal differences between these two codes, mainly in the calculation of the Mie coefficients, the same results were obtained with either code.

For the purposes of these calculations, the scattering particle was treated as a homogeneous sphere of diameter, d, and uniform relative refractive index, m, illuminated with unpolarized light of wavelength, λ, in the surrounding medium ($\lambda = \lambda_{vac}/m_2$). For biological cells in aqueous suspension, the value of m was taken as 1.05, consistent with that assumed by other investigators (1–4, 7).

RESULTS

Curves of the scattering intensity as a function of scattering angle based on all three methods are shown in Fig. 1 for the case $\alpha = 70$, $m = 1.05$. In the Hodkinson ap-

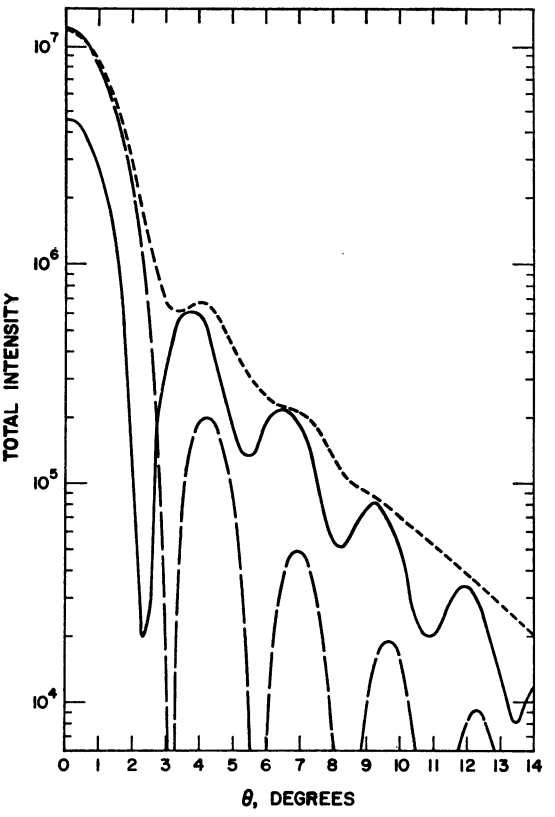

FIGURE 1 Total (both polarizations) Mie scattering intensity (———) as a function of the scattering angle, θ, for $\alpha = 70$, $m = 1.05$. The Hodkinson model (- - - - -) and diffraction (— — —) results are shown for comparison.

proximation, the refraction and diffraction contributions are simply added; phase relationships are neglected. Consequently, the minima predicted by the Mie theory and diffraction are obscured.

It may also be noted from Fig. 1 that the Mie result at $\theta = 0°$ is only 0.4 of the Hodkinson or diffraction results. For this case, the phase difference ρ ($\rho = 2\alpha[m - 1]$) between a refracted and diffracted ray is 7.0, close to the value of 7.7 associated with first minimum in the extinction curve for m close to 1. The extinction maxima and minima are associated with constructive and destructive interference, respectively, between refracted and diffracted light (16). Destructive interference between refracted and diffracted light results in a reduction of the intensity in the forward direction. The effect of simply adding diffraction and refraction is most noticeable in the Hodkinson curve; the minima are virtually lost, since interference is not taken into account. For this reason, it can be shown that for $\rho < 3.5$ the shape of the forward scattering lobe based on the Hodkinson approximation is in error (17). With increas-

ing α and m, the Hodkinson and Mie results at small angles are in better agreement and approach the diffraction prediction. This is shown in Fig. 2, where the scattering intensity (i_{Mie}) at 0.5° is plotted as a function of α^3 for several values of m between 1.02 and 2.00. The solid line is the Fraunhofer diffraction result (i_{diff}). For $m \geq 1.10$, the Mie and diffraction results approach the same value for any $\alpha > 35$. As $m \rightarrow 1$, an oscillation in the Mie results becomes apparent, as shown in Fig. 2

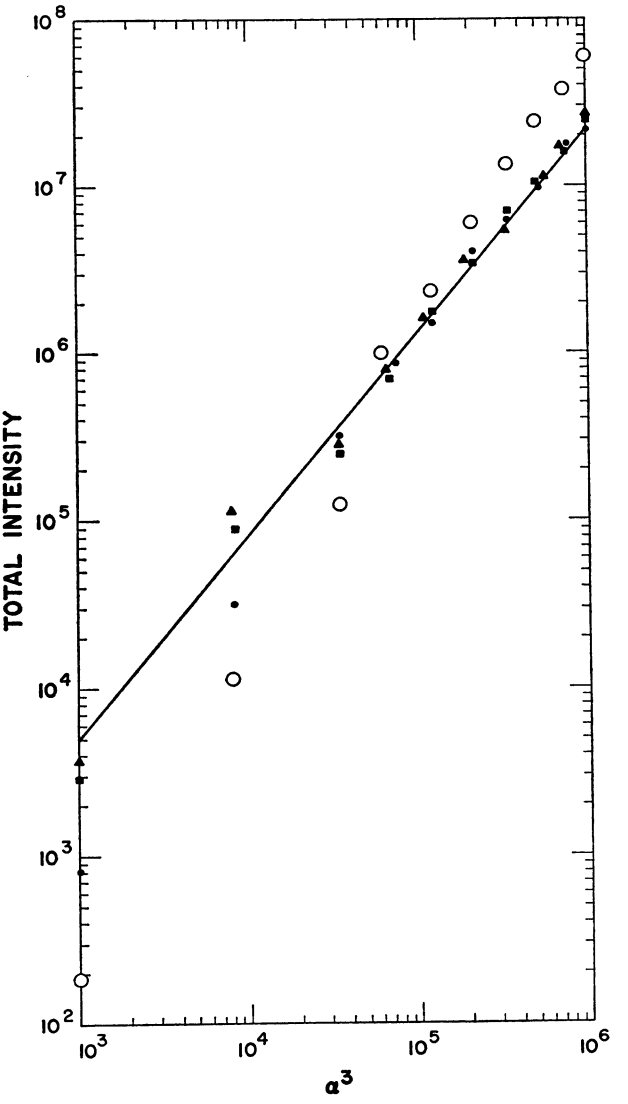

FIGURE 2 Total scattering intensity at $\theta = 0.5°$ as a function of α^3 for several refractive indices as calculated by DAMIE or DBMIE: $m = 1.02$ (○), $m = 1.10$ (●), $m = 1.20$ (▲), and $m = 2.0$ (■). The diffraction scattering intensity is shown as the solid line.

for $m = 1.02$ or in Fig. 3 for $m = 1.05$. For $m = 1.05$, the maximum in this oscillation occurs at $\alpha^3 = 6.4 \times 10^4$ and the minimum at $\alpha^3 = 4.5 \times 10^5$. Corresponding ρ values are 4.4 and 7.7, respectively. Since these correspond to the maxima and minima in the extinction curve, the oscillation is the result of interference effects between diffracted and transmitted (refracted) light as described above. The same arguments apply to the case of $m = 1.02$, where the first minimum in the extinction occurs at $\alpha = 190$ or $\alpha^3 = 6.9 \times 10^6$. In this case, the value of the intensity at 0.5° is greater than the diffraction prediction for almost the entire α range considered here, as shown in Fig. 2. The Hodkinson results for the intensity at 0.5° for the same α and m range show a behavior similar to that shown in Fig. 2, with the exception that the oscillation feature for small m is lacking. With increasing α and m, the Hodkinson results

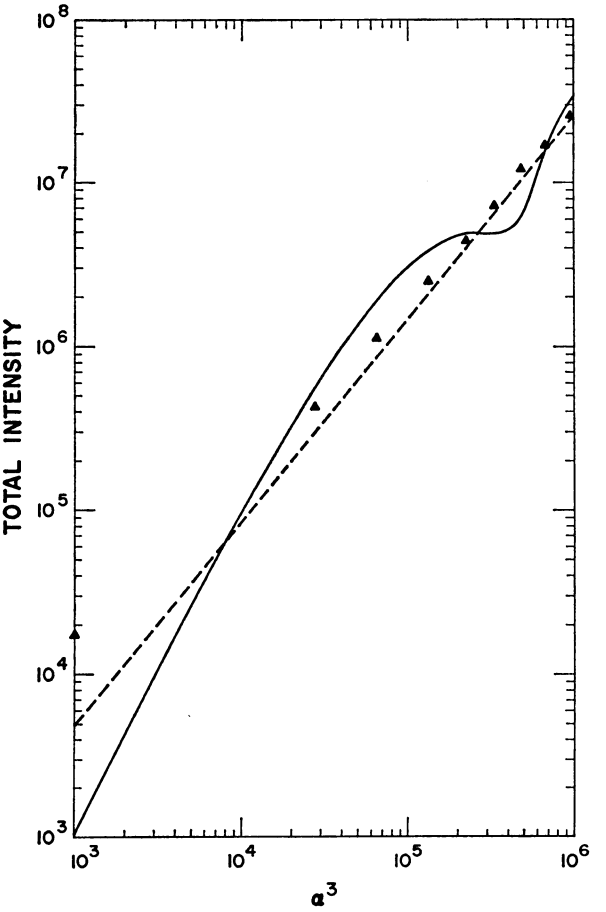

FIGURE 3 Total scattering intensity at $\theta = 0.5°$ calculated with the codes DAMIE and DBMIE for $m = 1.05$ as a function of α^3 (———). Also shown are the equivalent Hodkinson result (▲) and diffraction result (– – – –).

also approach the diffraction prediction of intensity proportional to $\alpha^{3.3}$ at a scattering angle $\theta = 0.5°$.

DISCUSSION

The results given above point out several advantages associated with measuring the scattering at 0.5°. Here one is measuring scattering very near the forward direction. The intensity at 0.5°, as calculated by Mie theory, is at least 84% of the intensity at 0°. Details of $i_{\text{Mie}}(0.5°)/i_{\text{Mie}}(0°)$ are given in Table I for the α range considered here. In actual practice, one measures the scattering between two angles (θ_1 and θ_2); the actual light-scattering signal is then the intensity integrated between θ_1 and θ_2. As θ increases, $i_{\text{Mie}}(\theta)$ decreases and the signal will be weighted more by the smaller angle scattering. If $\theta_1 = 0.5°$ and $\theta_2 = 2.0°$, as in the photometer we describe, Mie and diffraction calculations of the intensity integrated between these angles exhibit a behavior similar to that shown in Figs. 2 and 3. This point is made in Table I, where i_{Mie} (2.0°)/i_{Mie} (0.5°) is given for comparison. For a typical mammalian cell, $\alpha = 70$, i_{Mie} (0.5°) is about 25 times i_{Mie} (2.0°).

Although there are differences between Mie theory and the Hodkinson approach, calculations based on both methods indicate that the scattering signal at 0.5° is approximately proportional to volume for biological cells with $m = 1.05$, predicting that small angle light-scattering measurements will yield information on the volume distribution of a population of cells. The greater sensitivity of the Mie results to m does suggest that absolute volumes of low refractive index particles may not be easily obtained by simple comparison of the 0.5° light-scattering signal with that from a different particle of known diameter (e.g. plastic microspheres). Our model considers cells as homogeneous spheres of uniform refractive index, and this is somewhat unrealistic. All cells in a population are not spherical. The structure of a cell is certainly more complicated than assumed here; the concept of a mean refractive index for all cells in a population may be an oversimplification (18).

The next degree of complexity for a cell model would regard a cell as a nearly spherical particle with a cytoplasm of one refractive index and some major inclusion (nucleus, etc.) of a second refractive index. For example, the refractive index of the cytoplasm of the developing spermatid of *Locusta migratoria* is 1.3535 ($m = 1.018$)

TABLE I

RATIOS $i_{\text{Mie}}(0.5°)/i_{\text{Mie}}(0°)$ AND $i_{\text{Mie}}(2.0°)/i_{\text{Mie}}(0.5°)$ CALCULATED FOR $m = 1.05$

Ratio/α	10	20	30	40	50	60	70	80	90	100
$i_{\text{Mie}}(0.5°)/i_{\text{Mie}}(0°)$	0.998	0.994	0.985	0.972	0.952	0.921	0.872	0.870	0.868	0.843
$i_{\text{Mie}}(2.0°)/i_{\text{Mie}}(0.5°)$	0.99	0.90	0.78	0.63	0.44	0.22	0.04	0.06	0.08	0.04

and that the included *Nebenkern* have a refractive index of 1.3760 ($m = 1.035$ [19]). Both of these m values are lower than the $m = 1.05$ assumed here. Also, the refractive index of the cytoplasm of *Euglena* and human oral epithelial cells has been reported to be 1.37 ($m = 1.03$ [20]).

If $m = 1.05$ is an overestimate for living cells and a value of 1.02 to 1.03 is more typical, the small angle light-scattering behavior should be similar to that shown in Fig. 2 for $m = 1.02$. Here i_{Mie} is 1.5–3.0 i_{diff} for $\alpha > 40$. However, the scattering signal is nearly proportional to $\alpha^{3/3}$ over most of the α range, again indicating the possibility of obtaining approximate volume distribution information.

Changes in refractive index of locust (21) and grasshopper (22) spermatocytes during meiotic division have been reported, suggesting that there are changes in refractive index associated with various types of cellular activity (20). All of these factors mentioned above affect biological variability so that scattering would be described not by a single intensity curve but by a band of such curves. These factors would tend to smooth out some of the fine structure oscillation present in the Mie curves.

From Fig. 2 it is evident that the Mie intensity approaches the diffraction intensity under the conditions of increasing α and increasing m. An increase of α may be accomplished by illumination with light of a shorter wavelength. Fixing cells increases their refractive index. Crossmon (23) reported that human prostate gland sections fixed in formalin had a refractive index of 1.51–1.52 ($m \sim 1.14$). Exfoliated vaginal squamous (24) and epithelial cells (25) have a refractive index of 1.54 ($m = 1.16$). In this case, cells should behave like plastic microspheres. In Fig. 2, $i_{\text{mie}} \simeq i_{\text{diff}}$ for $m > 1.1$. For fixed cells, absolute volume as well as volume distributions should be obtained by calibration with a convenient particle of $m > 1.1$.

Variation of scattering signal at small angles with m can be minimized by reducing the amount of light refracted through the cell (for example by treatment with a stain that absorbs near the illuminating wavelength). Similarly, illumination with 260 nm light, where nucleic acids are known to adsorb, should also reduce the transmission. The Mie analysis permits use of a complex refractive index, $m = n_1 - in_2$, thus accounting for absorption. The value of n_2 was chosen so that transmission was 14% for all values of α considered. The results for $\theta = 0.5°$ are shown in Fig. 4. For all m, $i_{\text{Mie}} \simeq i_{\text{diff}}$, and absolute volume information should be obtainable.

We may conclude from this theoretical treatment that small angle light scattering is expected to yield relative volume distribution information on living mammalian cells. More accurate and more absolute volume results are predicted if cells are fixed or stained or if untreated cells are illuminated at shorter wavelengths, preferably where considerable absorption occurs. Experimental investigation of the predictions of this theoretical analysis is underway and will be reported on in a subsequent paper.

The authors wish to thank Dr. W. H. Beattie and Dr. M. A. Van Dilla for helpful discussions. This work was performed under the auspices of the U. S. Atomic Energy Commission.

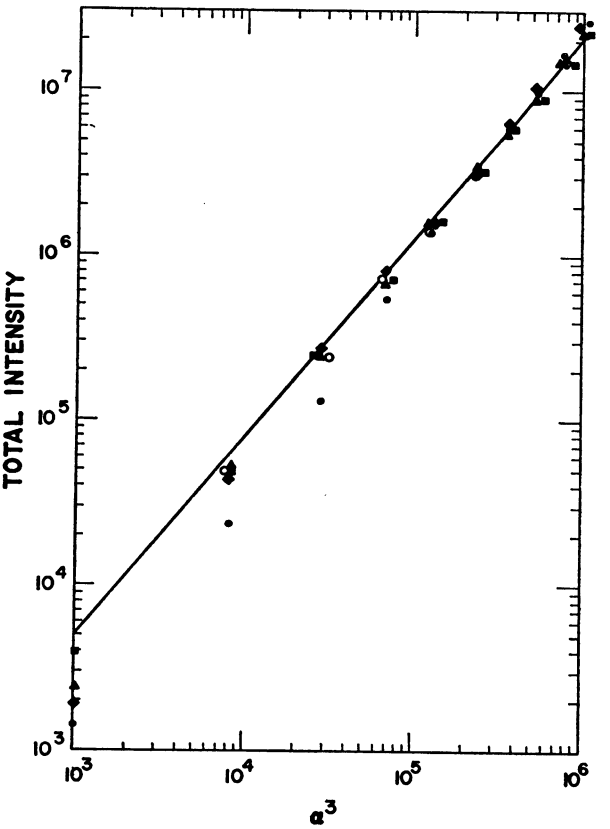

FIGURE 4 Total Mie scattering at $\theta = 0.5°$ for complex refractive index $m = n_1 - in_2$. The value of n_2 was chosen so that transmission through the particles was 14% for all α ($0.008 \leqq n_2 \leqq 0.1$). The real refractive indices shown are $n_1 = 1.02$ (●), $n_1 = 1.07$ (◆), $n_1 = 1.10$ (▲), and $n_1 = 1.20$ (■). Several values are also given for $n_1 = 2.00$ (○). The contribution from diffraction alone is shown as the solid line.

Received for publication 16 September 1969 and in revised form 5 March 1970.

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