ANOMALOUS DIFFRACTION APPROXIMATION TO THE LOW-ANGLE SCATTERING FROM COATED SPHERES

A Model for Biological Cells

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ABSTRACT The anomalous diffraction approximation has been used in an attempt to account for the low-angle scattering from composite spheres. These are used as models for biological cells. Solutions have been obtained for both thinly and thickly coated spheres.

INTRODUCTION

Recently there has been interest in the use of light scattering, and in particular low-angle light scattering, as a means of studying cells in suspension. Potential uses of the method are the sizing of cells, the identification of cells, the observation of internal cellular structure, and the monitoring of changes in cells. It has been common practice to use homogeneous spheres or coated spheres as models for cells. A comprehensive description of the low-angle scattering has been obtained by using the Mie theory for both homogeneous (1–4) and coated spheres (5–8). A number of workers have used the Rayleigh-Gans-Debye (RGD) approximation (9–13), chiefly for the smaller cells such as bacteria. This method has the advantage of not being confined to the assumption of spherical shape. It has the disadvantage of requiring that the scatterers be "small" (i.e. with respect to the wavelength of the scattered light). Other approximations have included the use of diffraction formulae (10, 11) and a combination of diffraction and refraction formulae (14). The former are strictly applicable only to "large" particles, whilst the latter may be considered to apply to intermediate sizes.

Little use appears to have been made of the approximation entitled "anomalous diffraction" by van de Hulst (15). This method is valid for scattering particles that are not optically dense, but are large enough such that significant phase differences occur for rays penetrating the scatterer. Anomalous diffraction has been employed in discussing the turbidity of cell suspensions (4). Historically, anomalous diffraction has been applied only to the scattering from spherical or orientated cubic bodies (15, 16). Recently Latimer (17) has used a combination of Mie theory and anomalous diffraction to compute the scattering patterns of ellipsoidal bodies.

If the cell and cell contents have refractive indices close to that of the surrounding medium, and if the cell can be modeled as a homogeneous or coated sphere, then pure anomalous diffraction seems ideally suited to describing the low-angle scattering of cells and microorganisms. The method reduces to the RGD formulation for small scatterers and to the diffraction formulae for large scatterers. It therefore appears to provide a better description of the intermediate-size region than the simple addition of diffraction and refraction terms.

DERIVATIONS

In this note we shall attempt to derive simple analytical solutions for the low-angle scattering from coated spheres using the anomalous diffraction approximation. We shall employ the simple model of two homogeneous and isotropic concentric spheres of radii a_1 and a_2, with a_1 greater than a_2 and refractive indices n_1, n_2 embedded in a homogeneous and isotropic medium of refractive index n (Fig. 1). We assume that $n \approx n_1 \approx n_2$ to neglect refraction and reflection at the interfaces between different media. The scattered intensity depends on the square of the modulus of the complex amplitude $S(\theta)$ of the scattered wave for scattering at an angle θ to the forward direction of the incident beam. Following the notation employed by van de Hulst (15) for a single sphere, we have for the composite sphere

$$S(\theta) = \frac{X_1^2}{2\pi} \int_{\phi=0}^{2\pi} \int_{\tau_1=0}^{\pi/2} (1 - e^{-i\delta}) e^{-iZ_1 \cos\tau_1 \cos\phi} \cdot \sin\tau_1 \cos\tau_1 \, d\tau_1 d\phi, \quad (1)$$

where $Z_1 = X_1\theta = (2\pi/\lambda)a_1\theta$, λ is the wavelength of the scattered light in the sur-

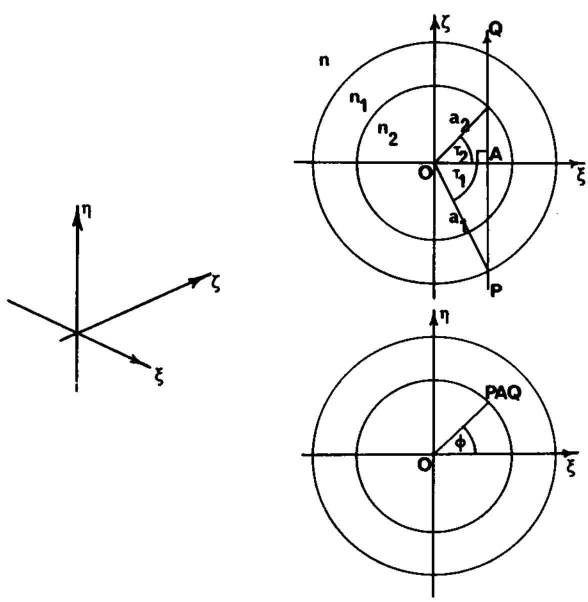

FIGURE 1 Path of a representative light ray (P to Q) through a concentric coated sphere, positioned about the origin of the coordinate set.

rounding medium, and δ is the phase lag introduced at Q through traversing the composite sphere along PQ. The angle ϕ defines the orientation of OA in the plane that contains O and is perpendicular to the incident beam direction. Each of the constituent media contributes to the total phase lag experienced by the beam, hence we may write: $\delta = \rho_2 \sin \tau_2 + \rho_1 \sin \tau_1$ where $\rho_1 = (4\pi/\lambda)a_1(n_1 - n)/n$, $\rho_2 = (4\pi/\lambda) \cdot a_2(n_2 - n_1)/n$. It is the inclusion of the term $\rho_2 \sin \tau_2$ over the range $0 < \tau_2 < 90°$ that leads to difficulties in evaluating the integrals in Eq. 1. It is convenient to divide the integration into two parts and introduce the parameter $Z_2 = X_2\theta = (2\pi/\lambda)a_2\theta$, whence

$$S(\theta) = S_1(\theta) + S_2(\theta)$$

$$= \frac{X_1^2}{2\pi} \int_{\phi=0}^{2\pi} \int_{\tau_1=0}^{\cos^{-1}(a_2/a_1)} (1 - e^{-i\rho_1 \sin\tau_1}) e^{-iZ_1 \cos\tau_1 \cos\phi} \cdot \sin\tau_1 \cos\tau_1 \, d\tau_1 d\phi$$

$$+ \frac{X_2^2}{2\pi} \int_{\phi=0}^{2\pi} \int_{\tau_2=0}^{\pi/2} (1 - e^{-i(\rho_2 \sin\tau_2 + \rho_1 \sin\tau_1)}) \cdot e^{-iZ_2 \cos\tau_2 \cos\phi} \cdot \sin\tau_2 \cos\tau_2 \, d\tau_2 d\phi. \quad (2)$$

We have used the relations

$$a_1 \cos\tau_1 = a_2 \cos\tau_2$$
$$a_1^2 \cos\tau_1 \sin\tau_1 d\tau_1 = a_2^2 \cos\tau_2 \sin\tau_2 d\tau_2, \quad (3)$$

which are valid for $0 < \tau_2 < 90°$. Integrating with respect to ϕ (see Appendix) and introducing $\gamma_1 = (\pi/2 - \tau_1)$ and $\gamma_2 = (\pi/2 - \tau_2)$, we have

$$S_1(\theta) = X_1^2 \int_{[(\pi/2)-\cos^{-1}(a_2/a_1)]}^{\pi/2} (1 - e^{-i\rho_1 \cos\gamma_1}) J_0(Z_1 \sin\gamma_1) \sin\gamma_1 \cos\gamma_1 \, d\gamma_1, \quad (4a)$$

$$S_2(\theta) = X_2^2 \int_0^{\pi/2} (1 - e^{-i(\rho_2 \cos\gamma_2 + \rho_1 \cos\gamma_1)}) \cdot J_0(Z_2 \sin\gamma_2) \sin\gamma_2 \cos\gamma_2 \, d\gamma_2. \quad (4b)$$

For arbitrary values of a_1 and a_2, it is necessary to eliminate γ_1 from Eq. 4*b* by using Eq. 3. Unfortunately equations 4*a* and 4*b* then need to be evaluated numerically. In such an eventuality, the anomalous diffraction method offers no advantage over the complete Mie theory computations. However, comparatively simple solutions may be obtained to the anomalous diffraction equations in two particular cases, which may represent realistic models for certain types of biological cells.

Thinly Coated Sphere ($a_1 \approx a_2$)

In this case $\cos\gamma_1 \approx \cos\gamma_2$ so that

$$S_2(\theta) \approx X_2^2 \int_0^{\pi/2} (1 - e^{-i\rho_0 \cos\gamma_2}) J_0(Z_2 \sin\gamma_2) \sin\gamma_2 \cos\gamma_2 \, d\gamma_2 \quad (5a)$$

with $\rho_0 = \rho_1 + \rho_2$. The factor $S_1(\theta)$ may be replaced by the residue

$$R_a \approx X_1^2 \cos^{-1}\left(\frac{a_2}{a_1}\right)\left(1 - \exp\left[-i\rho_1 \sqrt{\frac{a_1 - a_2}{2a_1}}\right]\right) J_0(Z_1) \sqrt{\frac{a_1 - a_2}{2a_1}}. \qquad (5b)$$

The expression for $S_2(\theta)$ is now equivalent to that for a homogeneous sphere of radius a_1 and refractive index equal to $\{n_2 - (t/a_1)(n_2 - n_1)\}$ where t is the thickness of the thin coat. (i.e. $t = (a_1 - a_2)$). Thus following the method employed by van de Hulst for the case of a homogeneous sphere, we can now solve $S_2(\theta)$ for the cases of large or small ρ_0. This solution can be written in terms of the series expansions I_a, I_b, and I_c, as defined in the appendix by Eqs. A2, A4, and A5. Thus for small ρ_0, as in the case of small particles

$$S(\theta) = X_2^2 I_b(\rho_0, Z_2) + R_a + iX_2^2 I_a[\rho_0, y(0,2)],$$

and for large ρ_0, as with large particles

$$S(\theta) = X_2^2 I_c[\rho_0, Z_2, y(0,2)] + R_a + iX_2^2 I_a[\rho_0, y(0,2)].$$

The quantity $|S(\theta)|^2$ describes the angular dependence of the scattered intensity for a single particle.

Thickly Coated Sphere ($a_1 \gg a_2$)

In this case over the region defined by $0 < \tau_2 < \pi/2$ the value of $\cos\gamma_1$ is very close to unity. Hence the integrations simplify to the forms

$$S_2(\theta) \approx X_2^2 \int_0^{\pi/2} (1 - e^{-i(\rho_1 + \rho_2 \cos\gamma_2)}) J_0(Z_2 \sin\gamma_2) \sin\gamma_2 \cos\gamma_2 \, d\gamma_2,$$

and

$$S_1(\theta) \approx X_1^2 \int_0^{\pi/2} (1 - e^{-i\rho_1 \cos\gamma_1}) J_0(Z_1 \sin\gamma_1) \sin\gamma_1 \cos\gamma_1 \, d\gamma_1 + R_b,$$

where the residue R_b is given by the equation

$$R_b \approx X_1^2 \left\{\frac{\pi}{2} - \cos^{-1}\left(\frac{a_2}{a_1}\right)\right\}\{1 - e^{-i\rho_1}\} J_0\left(Z_1 \cdot \frac{a_2}{2a_1}\right) \cdot \frac{a_2}{2a_1}.$$

The above integrals can now be rewritten in the form:

$$\begin{aligned} S_2(\theta) \approx X_2^2 \Bigg\{ &(1 - e^{-i\rho_1}) \int_0^{\pi/2} J_0(Z_2 \sin\gamma_2) \sin\gamma_2 \cos\gamma_2 \, d\gamma_2 \\ &+ e^{-i\rho_1} \int_0^{\pi/2} (1 - \cos(\rho_2 \cos\gamma_2)) J_0(Z_2 \sin\gamma_2) \sin\gamma_2 \cos\gamma_2 \, d\gamma_2 \\ &+ ie^{-i\rho_1} \int_0^{\pi/2} \sin(\rho_2 \cos\gamma_2) \cdot J_0(Z_2 \sin\gamma_2) \sin\gamma_2 \cos\gamma_2 \, d\gamma_2 \Bigg\} \end{aligned}$$

$$S_1(\theta) \approx X_1^2 \int_0^{\pi/2} (1 - \cos(\rho_1 \cos\gamma_1)) \cdot J_0(Z_1 \sin\gamma_1) \sin\gamma_1 \cos\gamma_1 \, d\gamma_1 + R_b$$

$$+ iX_1^2 \int_0^{\pi/2} \sin(\rho_1 \cos\gamma_1) \cdot J_0(Z_1 \sin\gamma_1) \sin\gamma_1 \cos\gamma_1 \, d\gamma_1 .$$

Solutions to integrals of this form are indicated in the appendix. Three types of solution for these equations are possible depending on the magnitude of the parameters of ρ_1 and ρ_2.

For ρ_1 and hence ρ_2 small, we have

$$S(\theta) = X_1^2 I_b(\rho_1, Z_1) + R_b + iX_1^2 I_a[\rho_1, y(1,1)] + X_2^2(1 - e^{-i\rho_1}) \frac{1}{Z_2} J_1(Z_2)$$
$$+ X_2^2 e^{-i\rho_1} I_b(\rho_2, Z_2) + iX_2^2 e^{-i\rho_1} \cdot I_a[\rho_2, y(2,2)].$$

With ρ_1 and ρ_2 both large,

$$S(\theta) = X_1^2 I_c[\rho_1, Z_1, y(1,1)] + R_b + iX_1^2 I_a[\rho_1, y(1,1)]$$
$$+ X_2^2(1 - e^{-i\rho_1}) \cdot \frac{1}{Z_2} \cdot J_1(Z_2) + X_2^2 e^{-i\rho_1} \cdot I_c[\rho_2, Z_2, y(2,2)]$$
$$+ iX_2^2 e^{-i\rho_1} \cdot I_a[\rho_2, y(2,2)].$$

Finally for ρ_1 large and ρ_2 small,

$$S(\theta) = X_1^2 I_c[\rho_1, Z_1, y(1,1)] + R_b + iX_1^2 I_a[\rho_1, y(1,1)]$$
$$+ X_2^2(1 - e^{-i\rho_1}) \frac{1}{Z_2} \cdot J_1(Z_2) + X_2^2 e^{-i\rho_1} \cdot I_b(\rho_2, Z_2)$$
$$+ iX_2^2 e^{-i\rho_1} \cdot I_a[\rho_2, y(2,2)].$$

DISCUSSION

Thus, relatively simple series solutions to $S(\theta)$ can be obtained for the cases of thinly and thickly coated spheres. Thinly coated small sphere models could be used to describe the low-angle scattering from spherical bacteria such as *Serratia marcescens*, *Staphylococcus aureus*, or *Staphylococcus epidermidis*, from bacterial spheroplasts or L-forms.

Thinly coated large spheres provide models for at least two types of structures. These are: (*a*) Large cells with little internal features, but with optically significant cell walls or membranes. Red cell ghosts are typical of such systems. (*b*) Cells containing large internal features such as a large nucleus with little cytoplasm. Examples are erythroblasts or lymphocytes.

There seem to be few, if any, cells describable by the thickly coated sphere model. However, the equations for thinly coated and thickly coated spheres could allow one to estimate the angular regions most susceptible to changes in size of the internal and/or overall features of the cells. Such results may be useful in qualitatively interpreting scattering changes arising from either cell growth or the stimulation of such cells as lymphocytes.

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APPENDIX

The following equations represent solutions to integrals encountered in the text. The notation follows that employed by van de Hulst (15)

$$\frac{1}{2\pi}\int_0^{2\pi} e^{-iZ\cos\tau\cos\phi}\cdot d\phi = J_0(Z\cos\tau) \qquad (A1)$$

Where $J_0(Z\cos\tau)$ is a Bessel function of order zero and of argument $Z\cos\tau$, of the first kind

$$\int_0^{\pi/2} \sin(\rho_\alpha\cos\gamma)J_0(Z_\beta\sin\gamma)\sin\gamma\cos\gamma\cdot d\gamma$$

$$= \frac{\rho_\alpha}{y^2(\alpha,\beta)}\left(\frac{\pi y(\alpha,\beta)}{2}\right)^{1/2} J_{3/2}[y(\alpha,\beta)] = I_a[\rho_\alpha, y(\alpha,\beta)] \qquad (A2)$$

where for simplicity we write $y^2(\alpha,\beta) = \rho_\alpha^2 + Z_\beta^2$, $\alpha = 0,1,2$ and $\beta = 1,2$.

$$\int_0^{\pi/2} J_0(Z_\beta\sin\gamma)\sin\gamma\cos\gamma\, d\gamma = \frac{1}{Z_\beta}\cdot J_1(Z_\beta) \qquad (A3)$$

$$\int_0^{\pi/2} [1 - \cos(\rho_\alpha\cos\gamma)]J_0(Z_\beta\sin\gamma)\sin\gamma\cos\gamma\, d\gamma$$

$$= \rho_\alpha^2\cdot\frac{1}{Z_\beta^2}\cdot J_2(Z_\beta) - \frac{\rho_\alpha^4}{1\cdot 3}\cdot\frac{1}{Z_\beta^3}\cdot J_3(Z_\beta) + \frac{\rho_\alpha^6}{1\cdot 3\cdot 5}\cdot\frac{1}{Z_\beta^4}\cdot J_4(Z_\beta) + \cdots$$

$$= I_b(\rho_\alpha, Z_\beta) \qquad (A4)$$

when ρ_α is small (15) and for the case of ρ_α large (15), we have

$$\int_0^{\pi/2} (1 - \cos(\rho_\alpha\cos\gamma))J_0(Z_\beta\sin\gamma)\sin\gamma\cos\gamma\, d\gamma$$

$$= \frac{1}{Z_\beta}J_1(Z_\beta) + \frac{\rho_\alpha}{y^2(\alpha,\beta)}\left(\frac{\pi y(\alpha,\beta)}{2}\right)^{1/2} N_{3/2}[y(\alpha,\beta)]$$

$$+ \frac{1}{\rho_\alpha^2}\cdot J_0(Z_\beta) + \frac{1\cdot 3}{\rho_\alpha^4}\cdot Z_\beta\cdot J_1(Z_\beta) + \frac{1\cdot 3\cdot 5}{\rho_\alpha^6}\cdot Z_\beta^2\cdot J_2(Z_\beta) + \cdots$$

$$= I_c[\rho_\alpha, Z_\beta, y(\alpha,\beta)] \qquad (A5)$$

The function $N_n(Z)$ is defined by the relation

$$H_n^{(2)}(Z) = J_n(Z) - iN_n(Z), \qquad (A6)$$

where $J_n(Z)$ is a Bessel function of order n and of the first kind and $H_n^{(2)}(Z)$ is a Bessel function of order n of the second kind.

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