Complex refractive index of non-spherical particles in the vis-NIR Region – Application to *Bacillus Subtilis* spores

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A method for the estimation of optical constants, in the UV-Vis-NIR region, of non-spherical particles in a suspension at concentrations where multiple scattering is significant is presented. The optical constants are obtained by an inversion technique using the adding-doubling method to solve the radiative transfer equation in combination with the single scattering theories for modelling scattering by non-spherical particles. Two methods for describing scattering by single scattering are considered: the T-matrix method and the approximate but computationally simpler Raleigh-Gans-Debye (RGD)approximation.The method is then applied to obtain the optical constants of *bacillus subtilis* spores in the wavelength region 400-1200nm. It is found that the optical constants obtained using the RGD approximation matches those obtained using the T-matrix method to within experimental error.

OCIS codes: 290.3030; 290.4210; 290.5850; 290.7050.

Introduction

The complex refractive index ($m = n(\lambda) + ik(\lambda)$) can provide important information required for the control of processes involving industrial and biological suspensions. Also, for the estimating particle size and size distribution using light scattering methods, accurate values of the optical constants (complex refractive index) are required [1]. It is highly desirable to obtain the optical constants without submitting the samples to high dilutions. A method that utilizes the radiative transfer theory to model multiple scattering in combination with theories to describe single particle characteristics have been developed for extracting the complex refractive index of spherical particles in suspensions [2-4]. For most biological suspensions, the particles are not spherical and a theory to account for the particle shape has to be used.

We present a general method for the estimation of optical constants, in the UV-Vis-NIR region, of non-spherical particles in a suspension at concentrations such that multiple scattering is significant. The optical constants are obtained by an inversion technique using the adding-doubling method to solve the radiative transfer equation [5] in combination with the single scattering theories for modeling scattering by non-spherical particles. The method is then applied to obtain the optical constants of bacillus subtilis spores in the wavelength region 400-1200nm from measurements of total diffuse reflectance and transmittance using an integrating sphere set-up. Two methods for describing scattering by single scattering are considered: the T-matrix method [6-7] and the less accurate but computationally much faster Raleigh-Gans-Debye (RGD) approximation [8-9].

2. Method

The radiative transfer equation (RTE) is solved using the adding-doubling method to obtain simulated values of total diffuse reflectance (R_{tds}) and transmittance (T_{tds}). The inputs required for the calculations are the mean diameter and standard deviation (for polydisperse samples) of particles, particle shape, cuvette pathlength (ℓ) and the optical constants, $n_w(\lambda)$ and $k_w(\lambda)$, of water. In addition, initial guess values $n_0(\lambda)$ and $k_0(\lambda)$ of the optical constants of the particle have to be provided. These inputs are used to compute the single particle parameters - the absorption cross-section (σ_{abs}), the scattering cross-section (σ_{sca}) and the anisotropy factor g. The albedo $\alpha = \sigma_{sca}/(\sigma_{abs} + \sigma_{sca})$ and the optical depth $\tau = (\sigma_{abs} + \sigma_{sca})\ell$ are then computed and along with g form the inputs to the adding-doubling routine. The outputs from this routine (R_{tds} and T_{tds}) are then compared with the measured values (R_{tdm} and T_{tdm}):

$$\sum = abs(R_{tdm} - R_{tds}) + abs(T_{tdm} - T_{tds}) \quad (1)$$

The guess values are then updated and the iterations carried out until convergence ($\Sigma \le 1.0e-7$) is achieved. This iteration was carried out using the function "fmincon" of the MATLAB® Optimization toolbox. Previously, this inversion scheme was used to extract the optical constants of spherical particles [3-4]. The single particle parameters in those studies were computed using the exact Mie theory for spherical particles [3] and approximations to speed up the calculations [4]. In this study, the inversion scheme is extended to apply to non-spherical particles by implementing the T-matrix method and the RGD approximation to compute the single particle parameters for ellipsoids.

2.1 Particle scattering parameters using the T-matrix method

In the T-Matrix method [6-7], the incident and the scattering fields are written in terms of vector spherical functions $\mathbf{M}_{mn}$ and $\mathbf{N}_{mn}$:

$$\mathbf{E}^{inc}(\mathbf{r}) = \sum_{n=1}^{\infty} \sum_{m=-n}^{n} [a_{mn} \mathbf{RgM}_{mn}(\kappa \mathbf{r}) + b_{mn} \mathbf{RgN}_{mn}(\kappa \mathbf{r})] \qquad (2)$$

$$\mathbf{E}^{sca}(\mathbf{r}) = \sum_{n=1}^{\infty} \sum_{m=-n}^{n} [p_{mn} \mathbf{M}_{mn}(\kappa \mathbf{r}) + q_{mn} \mathbf{N}_{mn}(\kappa \mathbf{r})], \; |\hat{\mathbf{r}}| > r_0 \qquad (3)$$

Making use of the linearity property of the Maxwell's equations and its boundary conditions the coefficients of the scatterer field, **p** and **q** can be linearly related to the incident field coefficients **a** and **b**, by a transition matrix, (T-Matrix):

$$\begin{bmatrix} \mathbf{p} \\ \mathbf{q} \end{bmatrix} = \begin{bmatrix} \mathbf{T}^{11} & \mathbf{T}^{12} \\ \mathbf{T}^{21} & \mathbf{T}^{22} \end{bmatrix} \begin{bmatrix} \mathbf{a} \\ \mathbf{b} \end{bmatrix} \qquad (4)$$

The extinction and the scattering cross-section averaged over the uniform orientation distribution of a non-spherical particle are given by:

$$\sigma_{ext} = -\frac{2\pi}{\kappa^2} \mathrm{Re} \sum_{n=1}^{n_{max}} \sum_{m=-n}^{n} [T^{11}_{mnmn} + T^{12}_{mnmn}] \qquad (5)$$

$$\sigma_{sca} = \frac{2\pi}{\kappa^2} \sum_{n=1}^{n_{max}} \sum_{n'=1}^{n_{max}} \sum_{m=-\max(n,n')}^{\max(n,n')} \sum_{i,j=1,2} \left| T^{ij}_{mnmn'} \right|^2 \qquad (6)$$

where n_{max} is the dimension of the matrix **T**. The absorption cross-section is computed as:

$$\sigma_{abs} = \sigma_{ext} - \sigma_{sca} \tag{7}$$

The phase function in the T-Matrix method is the first element $(a_1(\theta))$ of the Stokes scattering matrix. The asymmetry factor $g = \langle \cos\theta \rangle$ in the T-Matrix method is computed by:

$$g = \frac{1}{2}\int_{-1}^{+1} a_1(\theta)\cos\theta d\theta \tag{8}$$

Due to computational considerations the phase function was calculated using the Henyey-Greenstein function. It has been shown [3], using the case of spherical particles, that the error introduced due to this approximation in the estimation of optical constants is small. The T-matrix method was implemented in Matlab® using the method described by Mischenko [6, 7, 10].

2.2 Particle scattering parameters using the RGD approximation

The RGD approximation applies when the following conditions are satisfied:

$$\left|\hat{n} - 1\right| << 1 \quad \text{and} \quad x\left|\hat{n} - 1\right| << 1 \tag{9}$$

where $\hat{n}$ is the relative refractive index of the particle with respect to the medium and x is the size parameter. Under these conditions, the differential scattering cross-section F_{un}, for un-polarized incident wave is given by [8-9]:

$$F_{un} = \frac{\kappa^4}{8\pi^2} V_p^2 (1 + \cos^2\theta)(m_r - 1)^2 P(\theta) \tag{10}$$

where m_r is the complex refractive index of the particle relative to that of the medium, $\kappa = 2\pi/\lambda_0$, λ_0 is the wavelength of incident radiation in free space, V_p is the volume of the particle which for a prolate or oblate ellipsoid is given by:

$$V_p = \frac{4}{3}\pi acb \tag{11}$$

where a, c and b are the dimensions of the particle along the three axes. The spores are modeled as prolate spheroids for which, a = b < c. The form factor for an ellipsoid of revolution is given by [8],

$$P(\theta) = \left\{\frac{3}{u^3}(\sin u - u\cos u)\right\}^2 \tag{12}$$

$$u = 2ka\sin\frac{\theta}{2}\left(\cos^2\beta + \frac{b^2}{a^2}\sin^2\beta\right)^{1/2} \tag{13}$$

$$\cos\beta = -\cos\alpha\sin\frac{\theta}{2} + \sin\alpha\cos\frac{\theta}{2}\cos\varphi \tag{14}$$

where α is the angle of the incident radiation. For perpendicular radiation $\alpha = \pi/2$ and for parallel incident radiation $\alpha = 0$. The form factor for randomly oriented particles can be computed using [8, 9]:

$$P(\theta)_{\text{randomly oriented}} = \int_0^{\pi/2} P(\theta)\sin\beta d\beta \tag{15}$$

The scattering and absorption cross-sections are given by,

$$\sigma_{sca} = \int_0^{2\pi}\int_0^{\pi} F(\theta, a, \hat{n}, \lambda)\sin\theta d\theta d\phi \tag{16}$$

$$\sigma_{abs}(\lambda) = \frac{4\pi n(\lambda)k(\lambda)V_p}{\lambda} \tag{17}$$

The phase function in the RGD approximation is given by,

$$p(\cos\theta) = \frac{1}{4\pi^2 a^4}\frac{V_p{}^2 x^4 (\hat{n}-1)^2 (1-\cos^2\theta)P(\theta)}{\sigma_{sca}} \tag{18}$$

The anisotropy factor, $g = <\cos\theta>$, is given by,

$$g = <\cos\theta> = \int_0^{2\pi}\int_0^{\pi} p(\cos\theta)\cos\theta\sin\theta d\theta d\phi \tag{19}$$

3. Experiments

Bacillus subtilis spores in water suspension were obtained from the Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University. The population of the spores in water suspension was 1.1×10^{10} cfu/ml (colony forming units per millilitre), where cfu is equivalent to the number of spores. Figure 1 shows a scanning electron micrograph (SEM) of the spores used in this study. In this figure, it can be seen that the spores have a rod shape which can be simulated as a prolate ellipsoid [11]. In order to obtain an estimate of the error in the extracted optical constants, two more samples were prepared by diluting the original suspension in de-ionised water to obtain samples with concentrations of 5.5×10^9 cfu/ml and 2.75×10^9 cfu/ml. The sizes of the spores were estimated by measuring the length and the width of 56 spores from several SEM pictures. The mean of the longest axes, $2*a_{mean}=1.39$ μm with standard deviation of s_a= 0.1µm and the mean of the width, 2*b_{mean}= 0.71µm, with a standard deviation of s_b=0.06µm. Given the narrow distribution, the particles were considered as being monodisperse.

Figures 2(a) and (b) show measurements of total diffuse reflectance and transmittance of the spores in suspension obtained at the three concentrations. The samples were measured in a 2 mm optical glass cuvette using a DRA-2500 accessory (single integrating sphere setup) attached to the Cary 5000i UV-VIS-NIR spectrophotometer over a wavelength range of 300 – 1200 nm.

4. Results and discussion

4.1 Optical constants of bacilis subtilis spores

The optical constants for *Bacillus subtilis* spores were estimated by applying the inverse method using both the T-Matrix approach and the RGD approximation for ellipsoidal particles to compute the albedo (W_0), optical depth (τ) and the anisotropy factor (g). The estimated values of $n(\lambda)$ and $k(\lambda)$ are shown in figures 3(a) and (b). The values reported were estimated as the mean value over three different concentrations ($1.1x10^{10}$, $5.5x10^9$ and $2.75x10^9$ cfu/ml) and the error bars are plotted as two times the standard deviation computed using the values estimated at the 3 concentrations. In these figures, for comparison purposes the optical constants published by Tuminello *et al* [12] for *Bacillus subtilis* spores in the region 200 nm to 2500 nm are also included. It is seen that the inversion scheme using the RGD approximation for single particle characteristics lead to $n(\lambda)$ and $k(\lambda)$ values which are similar to the ones obtained using the T-matrix method, with the differences between them being less than the experimental error.

Comparing the values obtained in this study with those of Tuminello *et al.*, from figure 3(a), it is seen that the inversion scheme used here results in $n(\lambda)$ values that are significantly higher. According to the current work, in the wavelength range 400-1200nm $n(\lambda)$ varies from 1.63-1.55 whereas Tuminello *et al.* obtained values around 1.52. The difference in the values estimated by the two methods is probably due to the differences in the methodology. Tuminello *et al* computed the imaginary part $k(\lambda)$ by measuring collimated transmittance and assuming that the scattering is negligible as:

$$k(\lambda) = \frac{\lambda \ln(T_{water} / T)}{4\pi f_v d} \quad (x)$$

where T_{water} is the transmittance of pure water and T is the transmittance of the spores suspension, f_v is the concentration, and d is the path length of the sample. The $n(\lambda)$ values were then computed using these values of $k(\lambda)$ through the Kramers-Kronig relations.

Another work that is found in the literature for estimating $n(\lambda)$ is that of Katz *et al.*[13] They estimated the refractive index of *Bacillus subtilis* spores using the Gaussian ray approximation of anomalous diffraction theory for two cases: assuming the spores as solid spherical particles and as two concentric non-absorbing spheres in the wavelength range from 400-1000nm. They reported a refractive index value of 1.55 for the first case, and 1.515 for the second.

From figure 3(b) it is seen that compared to the $k(\lambda)$ values reported by Tuminello *et al.*, the values obtained in this work are significantly smaller. Again these differences could be attributed to the different methodologies used.

The close agreement of $n(\lambda)$ and $k(\lambda)$ obtained through the RGD approximation compared to those obtained using the T-matrix is surprising especially since for the wavelength range and particle size considered the validity conditions for the RGD approximation viz. $|\hat{n}-1| << 1$ and $x|\hat{n}-1| << 1$ are not met. For the wavelength region considered in this work, we get $0.1831 \le |\hat{n}-1| \le 0.2065$ and $0.88 \le x|\hat{n}-1| \le 3.08$ from which it can be seen that the second condition is not met by the *bacillus subtilis* suspension. There could be two reasons for this agreement. One is that the error in calculating the single particle characteristics σ_{abs}, σ_{sca} and g using the RGD approximation is not large enough to cause significant deviations in the extracted optical constants despite the suspension not satisfying the validity conditions for the RGD approximation or it may be due to a cancellation of errors. In order to investigate the reason, the values of σ_{abs}, σ_{sca} and g obtained using the two methods were compared.

4.2 Comparison of single particle parameters computed by the T-matrix and RGD approximation

Figure 4(a) shows the absorption cross-section σ_{abs} for the *Bacillus subtilis* spores, modelled as prolate ellipsoids, using the T-Matrix and the RGD approximation for the range of size parameters encountered in this study. It is observed that the values obtained by the T-Matrix approach are much higher than the values given by the RGD approximation. The percent error in using the RGD approximation instead of the T-matrix method to compute the absorption cross-section is over 40% with the error increasing as the size parameter is increased (Figure 4(b)). In the case of scattering cross-section, it is seen from figure 5(a), that the RGD underestimates the magnitude of σ_{sca} for most of the size parameter range relevant for the current study. The error decreases with increasing size parameter (Figure 5(b)) and at a size parameter of about 13.5, there is a cross-over point where the RGD approximation starts to over-estimate the scattering cross-section. For the anisotropy factor, the RGD over-estimates the values over the entire size parameter range considered though the errors over the entire range is less than 5%.

This analysis indicates that the errors in using the RGD approximation in computing the single particle characteristics, especially the scattering and absorption cross-sections, are high for the range of size parameters encountered in this study. Both these parameters are under-estimated by the RGD approximation. The fact that despite such large discrepancies the estimated optical constants obtained using the RGD approximation does not significantly differ from those obtained using the T-matrix method suggests that the agreement is probably due to cancellation of errors. Another reason could be that the extracted optical constants are not very sensitive to the errors in the single particle parameters. However, this seems unlikely especially for the estimation of $k(\lambda)$. Studies with spherical particles [3] have shown that, when the RGD approximation is used instead of the exact Mie theory for computing particle characteristics, it resulted in very large errors (about 20%) in $k(\lambda)$ and the errors could be traced back to the errors in the computation of single particle characteristics.

5. Summary and conclusions

An inversion method to estimate the optical constants of non-spherical particles from measurements in the multiple scattering regime that uses the radiative transfer theory along with the T-matrix or the Rayleigh-Gans-Debye (RGD) approximation for describing single particle scattering characteristics was implemented. This method was applied to obtain the values of $n(\lambda)$ and $k(\lambda)$ of *Bacillus subtilis* spores in the wavelength region of 400-1200nm. It was seen that the inversion scheme using the RGD approximation for single particle characteristics lead to $n(\lambda)$ and $k(\lambda)$ values which are similar to the ones obtained using the T-matrix method, with the differences between them being less than the experimental error. This work leads to values of $n(\lambda)$ which are higher than those found in the literature [12,13].. The values of $k(\lambda)$ obtained in this work are significantly smaller than has been reported previously by [13].

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Figure captions

Figure 1 SEM of *Bacillus subtilis* spores in the suspension used in this study.

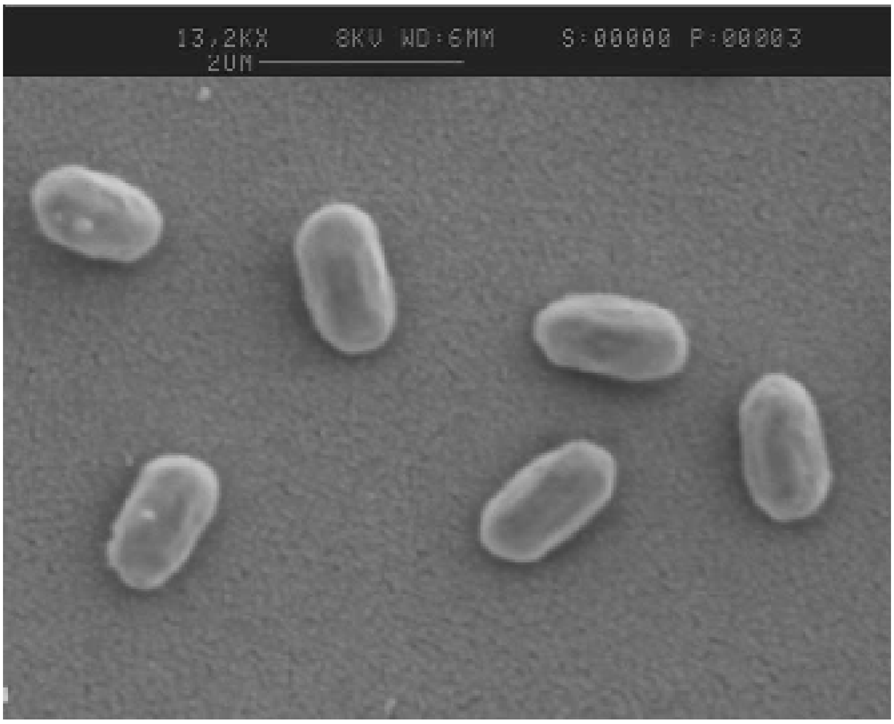
Figure 2. (a) Total diffuse reflectance and (b) Total diffuse transmittance for three different concentrations of *Bacillus subtilis* spores in water suspensions.

Figure 3 The optical constants (a) n, and (b) k, as a function of the wavelength of *Bacillus subtilis* spores in water suspensions estimated using the T-Matrix method and RGD approximation along with those reported by Tuminello *et al* [12].

Figure 4 (a) Absorption cross-section computed using the T-Matrix method and the RGD approximation for prolate ellipsoids in water; (b) Percent error in using the RGD approximation instead of the T-matrix method.

Figure 5 (a) Scattering cross-section computed using the T-Matrix method and the RGD approximation for prolate ellipsoids. (b) Percent error in using the RGD approximation instead of the T-matrix method.

Figure 6 (a) Anisotropy factor computed using the T-Matrix method and the RGD approximation for prolate ellipsoids; (b) Percent error in using the RGD approximation instead of the T-matrix method.



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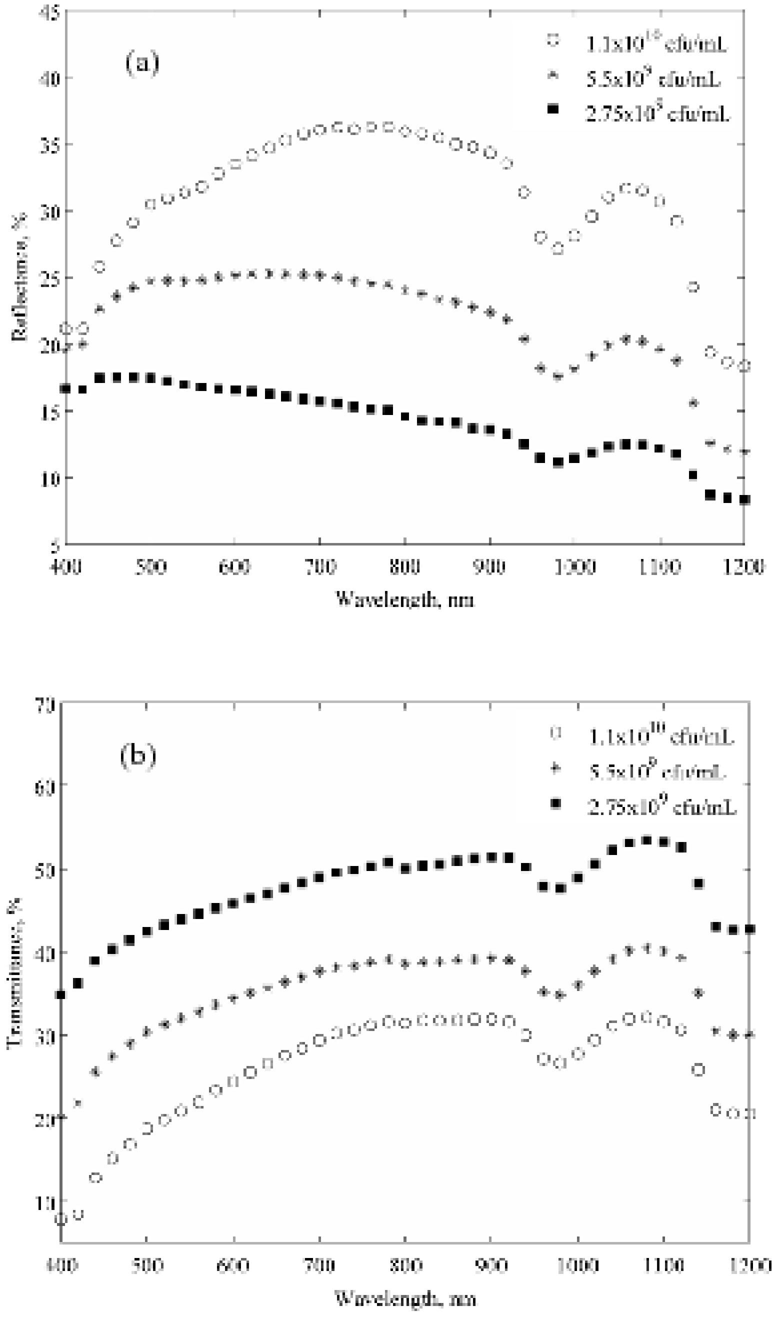

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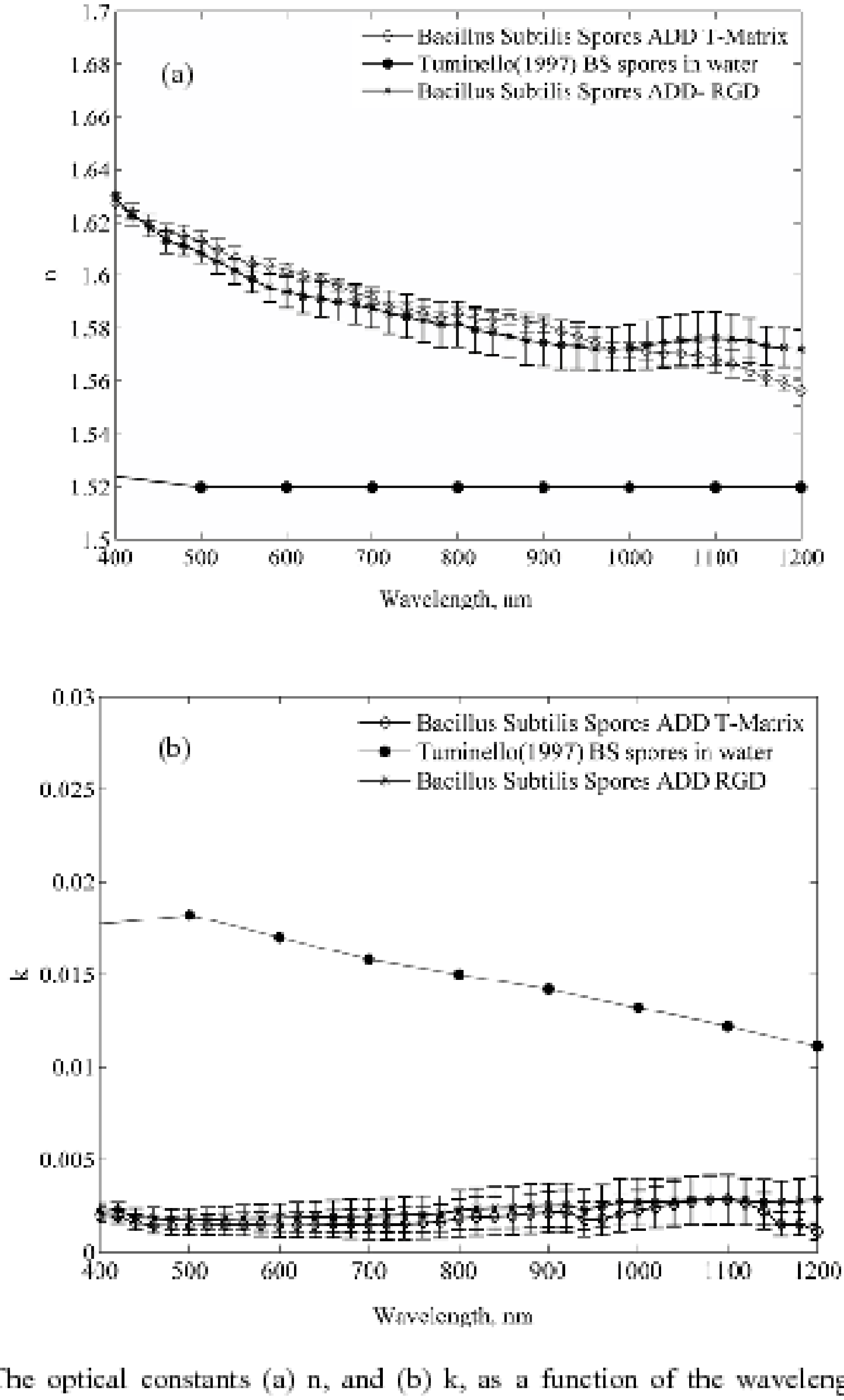

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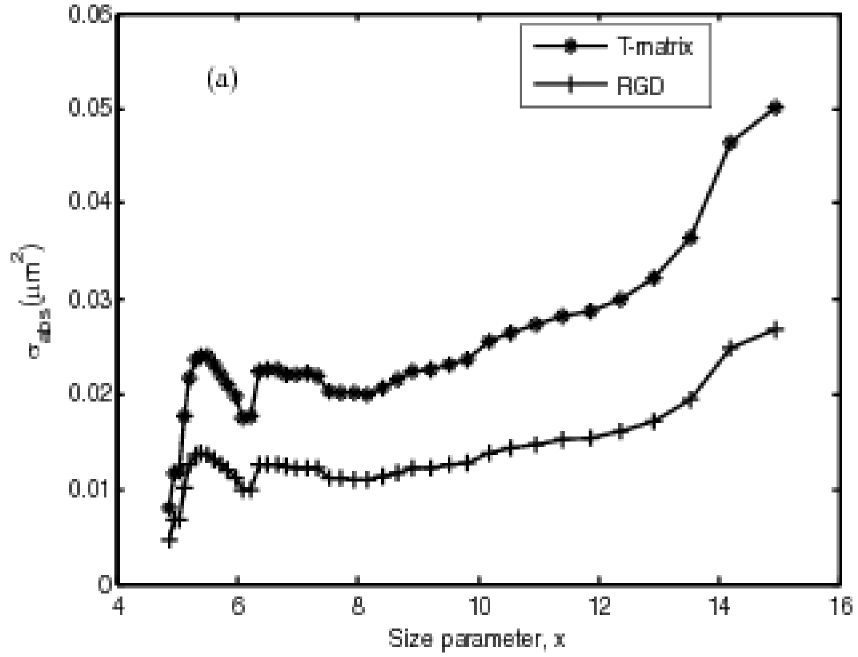

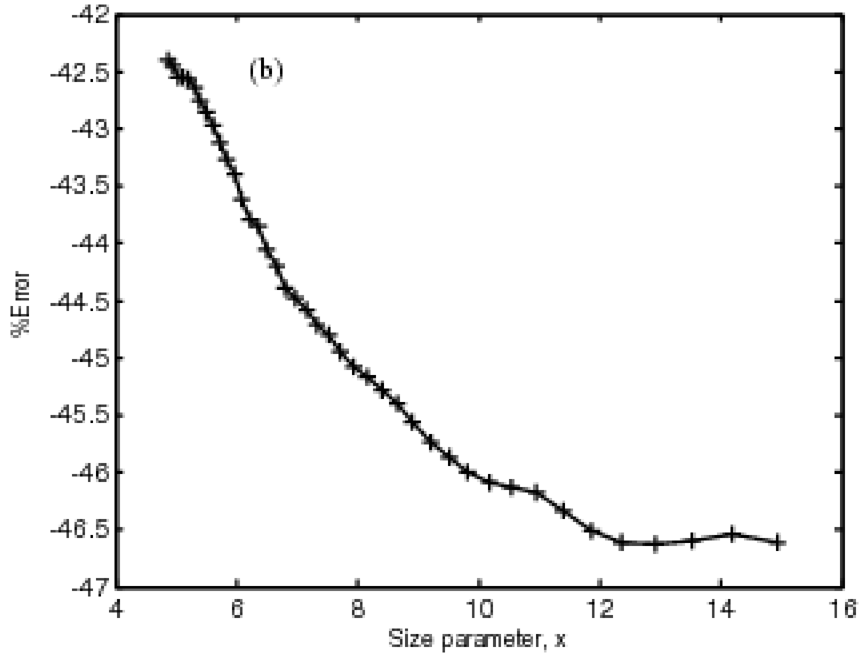

Figure 4 (a) Absorption cross-section computed using the T-Matrix method and the RGD approximation for prolate ellipsoids in water; (b) Percent error in using the RGD approximation instead of the T-matrix method.

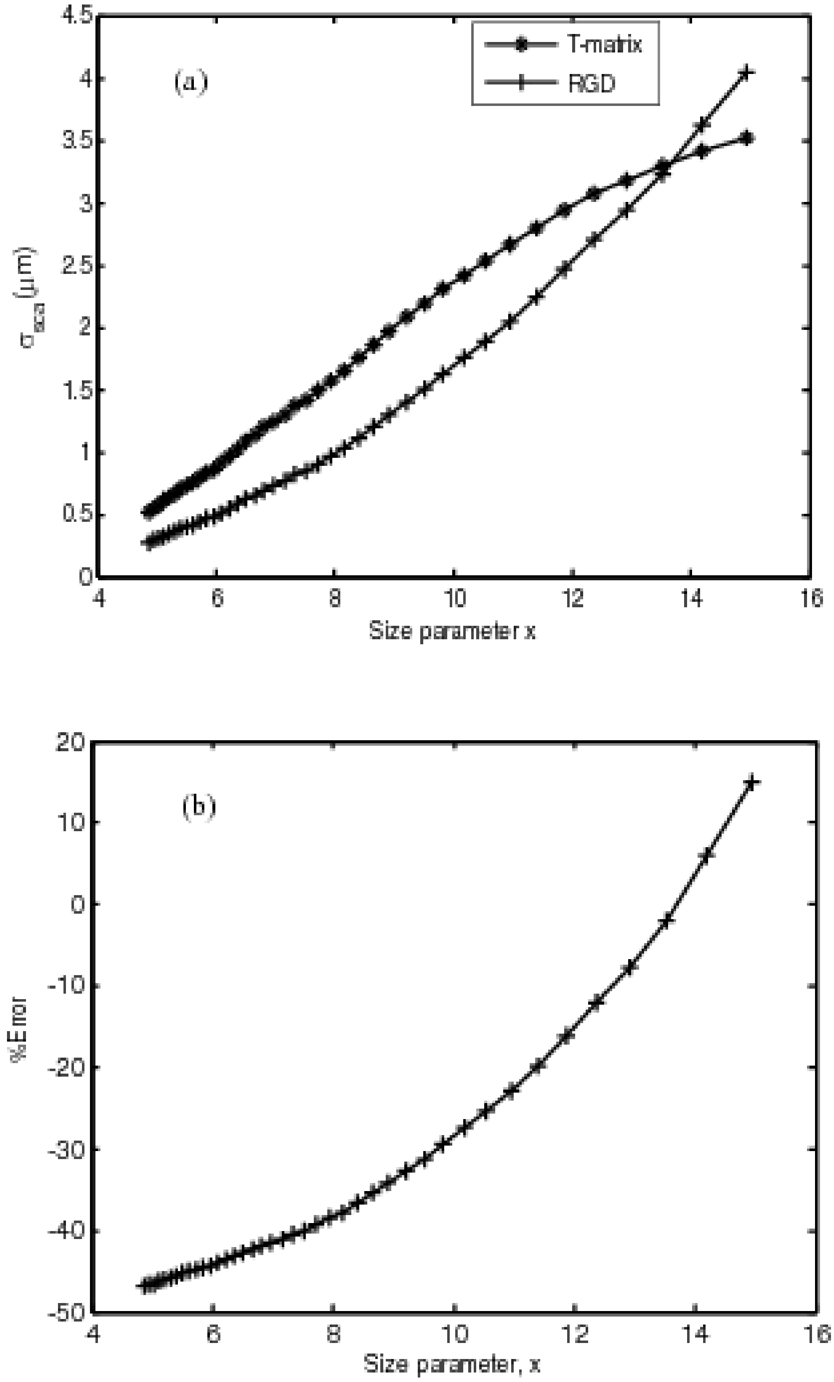

Figure 5 (a) Scattering cross-section computed using the T-Matrix method and the RGD approximation for prolate ellipsoids. (b) Percent error in using the RGD approximation instead of the T-matrix method.

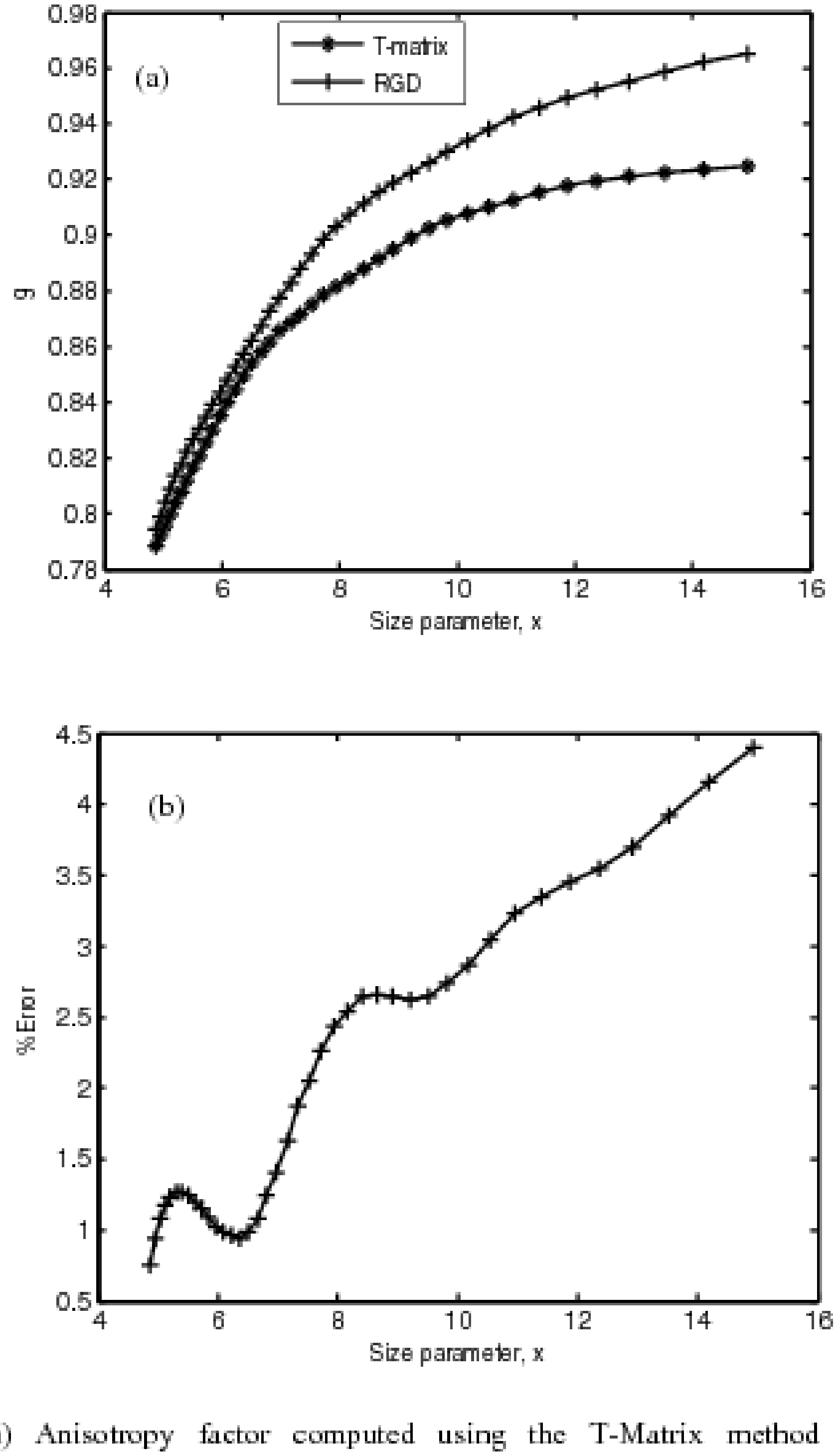

Figure 6 (a) Anisotropy factor computed using the T-Matrix method and the RGD approximation for prolate ellipsoids; (b) Percent error in using the RGD approximation instead of the T-matrix method.