SNO-STR-93-009

Light Scattering and Absorption due to Bacterial Activity in Water

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March 17, 1993

Abstract

There is a growing class of elementary particle detectors ("Large Water Čerenkov Detectors") which have a body of water (thousands of tons) as a sensitive medium. Particles are detected when they interact with the water and produce Čerenkov light, so detection efficiency relies on the transparency of the water. These detectors are active typically for many years and so biological activity is one of the means by which the transparency of the water may be reduced.

We present the results of a measurement of light scattering and absorption from a population of *E-coli* in water, which is used as a model for bacteria in general. The scattering and absorption can be separated by varying the refractive index of the medium using a solute of high molecular weight. We show that the results can be understood simply in terms of light scattering from small spheres (radius $\approx$ wavelength) with an effective refractive index n, plus a small amount of absorption, predominantly in the ultra-violet. We compare this scattering with Rayleigh scattering in pure water.

Introduction: Large Water Čerenkov Detectors

When elementary particles travelling faster than the local speed of light pass through a medium, they radiate light, predominantly in the blue and UV. This is called Čerenkov light after its discoverer [1]; it is the electromagnetic analogy of a sonic boom emitted by an aircraft travelling faster than the local speed of sound. In water the speed of light is 0.75 times that in a vacuum, and

so a body of water, instrumented with light sensors, forms an effective detector for any particle travelling faster than this. In elementary particle physics, such speeds are common.

Because water is cheap and transparent, it is possible to construct large Čerenkov detectors which can be used to search for rare processes like neutrino scattering and proton decay [2]. A typical size for this class of detectors is a few thousand tons, *i.e.* of order $10m$ radius. This size is large enough for the transparency of water to be affected by Rayleigh scattering (in the near UV) and potentially by biological activity. The former is an unavoidable consequence of the polar nature of the water molecule. The latter we will investigate here, in the hope of restricting its effects to be much less than that of Rayleigh scattering. Most of these detectors operate for many years with little intervention except for water purification, which has a long cycle time of weeks or months. There are factors in our favour: the detectors are operated cold and in the dark, and oxygen can be excluded from them by degassing the water and using an inert cover gas. However, these seemingly adverse conditions will not stop biological activity entirely [3]. Plastics are used extensively in detector construction and they leach nutrients (organic carbon, nitrogen, *etc.*) and dissolved oxygen into the water.

The detector we are most concerned with here is the Sudbury Neutrino Observatory (SNO) [2]. In this detector the active medium is *heavy* water, held in a $6m$ radius acrylic shell supported by ordinary "light" water. The light sensors are in the light water, $2.5m$ from the acrylic. So, a Čerenkov photon generated in the centre of the detector must traverse $6m$ of heavy water, $10cm$ of acrylic and $2.5m$ of light water to be detected. The wavelength distribution of detectable photons is from $300nm$ to $600nm$ with a maximum intensity at $380nm$. The cycle time of the water purification system is designed to be 25 days. The observatory is currently being built and is expected to start collecting data in mid-1995. A paper describing the biological implications of all the structural materials in both light and heavy water is presently in preparation [3]. The heavy water is not expected to cause biological problems; it is the light water component that we are primarily concerned with in this article.

In this work we will use *E-coli* as a convenient model for general bacterial activity in water particle detectors. We will also model bacteria more typical of those found in practice, which are somewhat smaller and tend to cluster. We note that in general light scattering is more important in this

application than light absorption. Particle detection relies on the *pattern* as well as the number of emitted Čerenkov photons, and this can be more easily disrupted by scattering than by the loss of a fraction of the photons.

Light scattering from small objects

To understand the scattering of light from *E-coli.*, it is important to note that the bacteria are approximately spherical with a radius $a \approx 0.5\mu m$. Thus the characteristic size is of the same order of magnitude as the wavelength λ of visible and near-ultraviolet light.

There are three classical approaches to light scattering [4, p.195]:

- Rayleigh scattering theory is valid for $ka \ll 1$, $nka \ll 1$, where $k = 2\pi/\lambda$.
- The Rayleigh-Gans-Debye approximation is valid for $n - 1 \ll 1$ and $2ka(n-1) \ll 1$.
- Anomalous diffraction is valid if $n - 1 \ll 1$ and $ka \gg 1$.

Unfortunately we have $n \approx 1.06 n_{water}$ (see below) and ka is of the order 10, so none of the above approaches works. We turn to the numerical tables of Wickramansinghe [5], compiled primarily for astrophysical usage. Wickramansinghe plots q_{sca} against x for small spheres, radius a, for various values of n; q_{sca} is the scattering cross section σ_{sca} divided by πa^2 and $x = ka$.

In figure 1 we show how the scattering cross sections vary according to $1/\lambda^m$, where m depends on bacterial size. Here we plot m as a function of the radius of the bacteria, assuming a spherical shape. The fit was done at $600nm$; in experimental data this is far from any absorption.

Wickramansinghe only plots scattering cross sections in increments of $\Delta n = 0.1$, and there is structure above $n = 1.1$, so it is not possible to deduce a useful dependency on n for $n < 1.1$ from them. However we can show experimentally that σ_{sca} is proportional to $(n/n_{med} - 1)^2$ for the x range in question. This is the same as in the Rayleigh formula for small n/n_{med}. Thus by varying n_{med} and making some assumptions about scattering and absorption, we can recover n.

The Effective Refractive Index of E-coli.

The solute we use for varying the refractive index of the medium must not enter the cells or change their volume, nor should it be be toxic [6, p.37]. We chose Ficoll-400 [7], a sugar polymer with a very high molecular weight (around 400,000) which discourages osmotic activity.

Ross explains various techniques for evaluating the refractive index of living cells and gives values of around 1.39 ($= 1.045 n_{water}$) for vegetative *B. cereus* and *B. megaterium* [6, p.133]. We chose a simple null technique in which the refractive index of the medium was varied until the transmission was maximized.

Physical Dimensions of Bacteria

The shape of *E-coli* is a rounded tube of length $2.45\mu m$ and diameter $0.55\mu m$ [8]. If we average the cross sectional area crudely using the three orthogonal axes of symmetry, we can represent the bacteria as spheres of radius $a = 0.50\mu m$.

The size of the bacteria growing in the detector is expected to be smaller than *E-coli*. Typical measurements of cells grown in simulated conditions indicate a length of about $1.2\mu m$ and a diameter of $0.7\mu m$. Thus we represent them in the same manner as above as spheres of radius $a = 0.43\mu m$, although we have observed variations of about a factor of two either way. Microscopic studies have also indicated that these cells tend to cluster in small groups, and we discuss the optical implications of this clustering below.

If we assume the bacteria are uniform spheres of refractive index $n = 1.064$ relative to the medium (see below), and radius a, we can deduce the following information:

$a\ (\mu m)$	Power Law m	$\sigma^{scatt}_{460nm}\ (\mu m^2)$	$A_{460nm}\ (10^9\ \text{Cells}/ml)$
0.2	2.6	0.00369	0.0277
0.3	2.1	0.0364	0.158
0.5	2.0	0.282	1.22
1.0	1.5	3.38	14.7

The value of m gives the wavelength dependence of the scattering: $1/\lambda^m$. An experimental determination of m gives a, which in turn gives σ_{scatt}. Now A will give the density ρ. Note $A = -log_{10}T$ (T is the fractional transmission) as per the usual biological definition, so for a cuvette length of $1cm$, and a cell density of ρ:

$A = \sigma(cm^2) \times \rho(cm^{-3})/2.303\ cm^{-1}$

The value of A is quoted above for $460nm$ by biological convention. The biological rule of thumb for *E-coli* is that $A = 1$ for $\rho = 10^9/ml$ at $460nm$. The peak of sensitivity for SNO occurs at $380nm$, so we can convert one to the other knowing m.

Results

We measure the combined scattering and absorption of a sample using a Perkin-Elmer Lambda-3B UV/VIS spectrophotometer. Any light scattered through more than 2° (*i.e.* essentially all the scattering) will not reach the light detector in the spectrophotometer, and so will be measured as absorbed.

To separate out the scattering and absorption we can fit this spectrum to Wickramansinghe's form above $400nm$, as expected from scattering theory. Below $400nm$ several absorption peaks as visible; these are the result of organic bonds in the proteins and nucleic acids in the bacteria. In the subsequent variation of n_{med}, we use this absorption to keep track of the concentration of the *E-coli*.

Now we vary n_{med}, using Ficoll-400, up to a maximum of $1.08n_{water}$, at which point the medium becomes so viscous as to be unusable. The scattering is normalized to concentration by fitting each spectrum and extracting the ratio Scattering($400nm$)/Absorption($250nm$) and plotting this against n_{med}/n_{water}. We assume there is no absorption at $400nm$. The results are shown in figure 2.

The curve is suggestive of a quadratic but the minimum is non-zero. We assume that this is because of a variation in refractive index within the *E-coli*, as noted in the microscopic work of Ross [6]. Thus there will never be a perfect optical match between medium and bacterium. We model this with a quadratic form with a minimum of n, but allowing a uniform spread in refractive index of Δn on either side of the central value. A value of $n = 1.064 \pm 0.002$ is extracted, with $\Delta n = 0.015 \pm 0.003$; so the bacterial refractive index varies between about 1.05 and 1.08 times that of water.

Using these data, we can make predictions with the model. For spheres of radius $a = 0.5\mu m$ with a refractive index of $n = 1.064$ relative to the medium, $\sigma_{sca} = .18 \times 10^{-8} cm^2$ at $460nm$. Thus the measured "absorption" for a $1cm$ cuvette in the spectrophotometer containing $2 \times 10^9 ml^{-1}$ of *E-coli* (density obtained from plate counting) will be:

$A_{theory} = 1.45$ in a typical mineral salt medium of $n = 1.014 n_{water}$, or

$A_{theory} = 2.44$ in pure water.

This is to be compared with our measured value of

$A_{expt} = 2.3$ in a mineral salt medium as above.

Both the theoretical and measured numbers compare reasonably well with the biologists rule of thumb that $A = 1$ per $10^9 ml^{-1}$ of bacterial density in a $1cm$ cuvette at $460nm$.

Figure 3 shows the combined scattering and absorption from a $1cm$ cell containing a sample of *E-coli.* with a density of $5 \times 10^7 ml^{-1}$. The refractive index of the medium (mineral salt solution) was found to be $n_{med} = 1.014 n_{water}$ using a refractometer.

One concern is that we noticed a small change in the shape of the absorption below $400nm$ as n_{med} was varied. This should not be so, and it may indicate that the Ficoll was changing the chemical structure of the bacteria. This change would introduce an additional uncertainty into our method of normalization.

Figure 4 shows the scattering and absorption from a $1cm$ cuvette containing a sample of cells cultured in pure water in the presence of urylon and polyethylene plastics. These plastics were chosen as they will be present in SNO in large amounts, urylon as the tank liner ($2000m^2$), and polyethylene as the cable jackets ($5400m^2$). Plastic samples of about $60cm^2$ surface were incubated in about $20ml$ of initially pure water at room temperature. Mineral salts were added and the mix was left for 13 days. Water was extracted for photometry. The curves on the logarithmic plot are straight lines in the visible region, which indicates very little absorption. Fitting for m indicates that the bacteria from the urylon are about $1\mu m$ in effective radius, and those from the polyethylene are $0.3\mu m$ in effective radius. The deviation from a straight line below $400nm$ shows the presence

of absorption due to DNA and proteins (the bump at about $260nm$ [9]) and possibly from organic leachates from the plastics.

The fits to our model will be affected by clustering. We can handle this by the following argument. If M cells accrete, the effective radius of the entity a_{eff} increases by $M^{1/3}$. The scattering cross section of the cluster is given by

$\sigma_{\text{eff}} = \pi a_{\text{eff}}^2 \times q_{sca}$

Now $q_{sca} \propto (ka)^m$ where m is typically 2, so

$\sigma_{\text{eff}} \propto a^4 \propto M^{4/3}$ for a given wavelength.

If there are N bacteria per unit volume, then the extinction factor (1/length) is given by

$\alpha = \sigma_{\text{eff}} \times N$, where $N \propto 1/M$, so

$\alpha \propto M^{1/3}$, which is a slow dependence.

We have observed M to be up to order 10, which would increase the expected scattering by a factor of about 2 for a given N. Photometrically we measure ρ, which is N/M, and the effective radius would be larger than for single cells. Biological cell counting gives ρ.

Figure 5 shows a comparison of Rayleigh scattering [10] with bacterial scattering. It can be seen that for bacterial densities of $10^5 ml^{-1}$ for $a = 0.3\mu m$, $10^4 ml^{-1}$ for $a = 0.5\mu m$, or $10^3 ml^{-1}$ for $a = 1\mu m$, bacterial scattering does not exceed Rayleigh anywhere in our sensitive wavelength region (it comes closest at the red end, $600nm$). At this level, bacterial absorption, which is only a few % of the bacterial scattering, is completely negligible. In any case, the purest water available exhibits scattering somewhat higher than Rayleigh levels for reasons other than biological activity [10].

Conclusion

We have found that by using a simple application of the theory of light scattering from small spheres, we can predict the following:

- The absolute amount of light scattered by *E-coli* in an aqueous medium to better than 30% for visible light.
- The form of light scattering by *E-coli* in an aqueous medium above $\lambda = 400nm$ to high precision.

The technique we have outlined can form the basis for a simple and accurate assessment of bacterial density and size based on light scattering. The amount of scattering above $400nm$ and its dependence on wavelength gives both the cell density and a rough estimate of cell size. Thus the effects of biological activity can be distinguished from molecular scattering and absorption which will have different wavelength dependences.

By extending our model of *E-coli* to the type of bacteria we expect to see growing in large water Čerenkov detectors, we conclude that a bacterial densities of $10^5 ml^{-1}$ for $a = 0.3\mu m$, $10^4 ml^{-1}$ for $a = 0.5\mu m$, or $10^3 ml^{-1}$ for $a = 1\mu m$, can exist in the water without compromising the efficiency of the detector.

Acknowledgements

The authors would like to thank Wendy Luther for her technical assistance. The work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

Figures

1. The scattering cross sections vary as $1/\lambda^m$ where m depends on bacterial size. Here we plot m as a function of the radius of the bacteria, assuming a spherical shape. The fit was done at $600nm$; in experimental data this is far from any absorption.

2. Variation in scattering with n_{med}. The scattering is normalized to concentration by fitting each spectra and extracting the ratio Scattering($400nm$)/Absorption($250nm$) and plotting this against n_{med}/n_{water}.

3. Scattering and absorption from a $1cm$ cell containing a sample of *E-coli.* with a density of 5 $\times 10^7 ml^{-1}$, as deduced from the scattering. The refractive index of the medium is $n_{med} = 1.014$. The line is the prediction of scattering theory, fitted in absolute value to the absorption/scattering ratio extracted from figure 2.

4. Scattering and absorption from a $1cm$ cuvette containing a sample of cells cultured in pure water in the presence of urylon and polyethylene plastics. The curves on this logarithmic plot are straight lines in the visible region, which indicates very little absorption. Fitting for m indicates that the bacteria from the urylon are about $1\mu m$ in radius, and those from the polyethylene are $0.3\mu m$ in radius. The deviation from a straight line below $400nm$ shows the presence of absorption due to DNA and proteins (the bump at about $260nm$) and possibly from organic leachates from the plastics.

5. Comparison of Rayleigh scattering with bacterial scattering. It can be seen that for a bacterial densities of $10^5 ml^{-1}$ for $a = 0.3\mu m$, $10^4 ml^{-1}$ for $a = 0.5\mu m$, or $10^3 ml^{-1}$ for $a = 1\mu m$, the bacterial scattering does not exceed Rayleigh anywhere in our sensitive wavelength region. The wavelength limits of the bacterial scattering calculations correspond to the limits of tabulation in Wickramansinghe [5].

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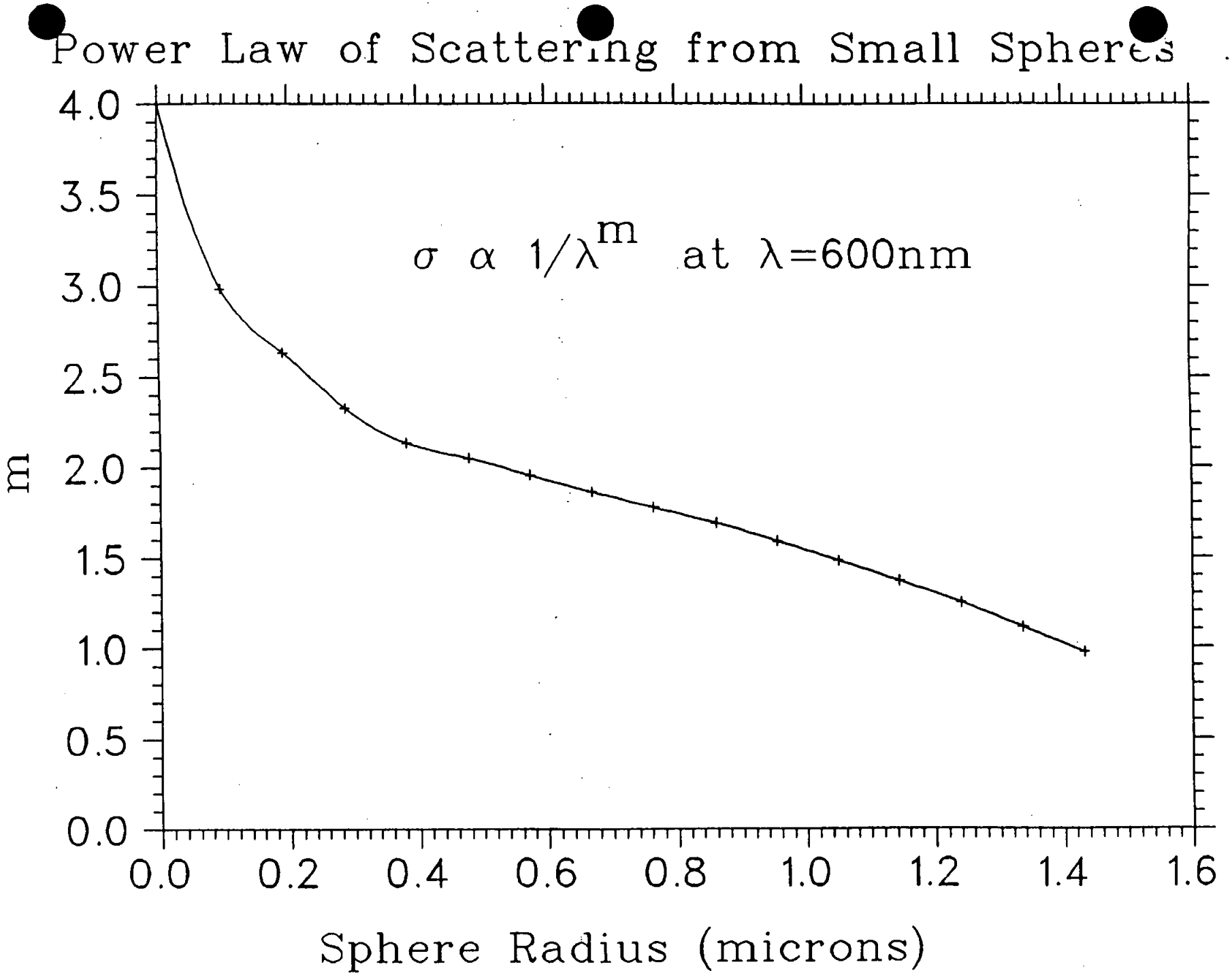
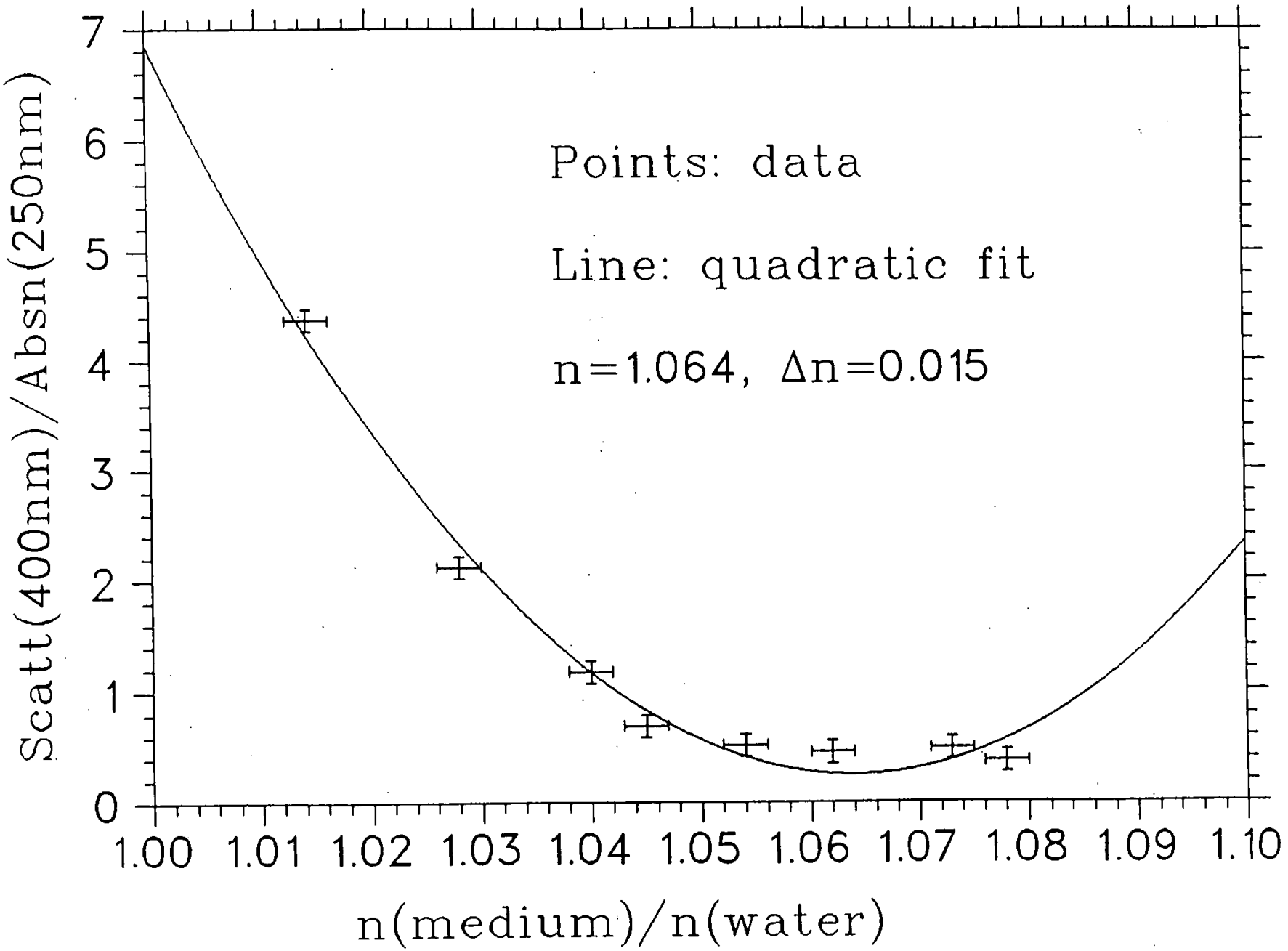
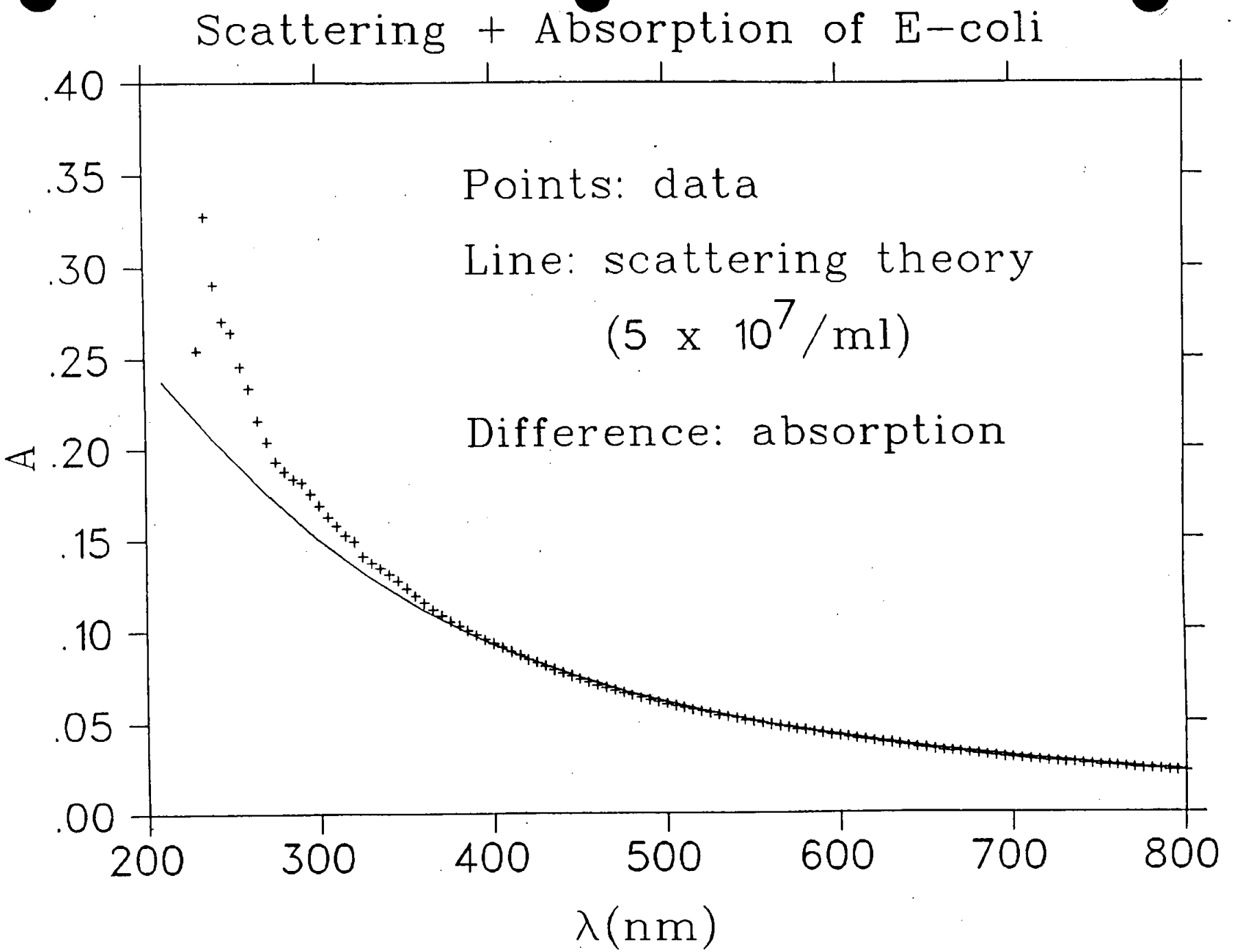
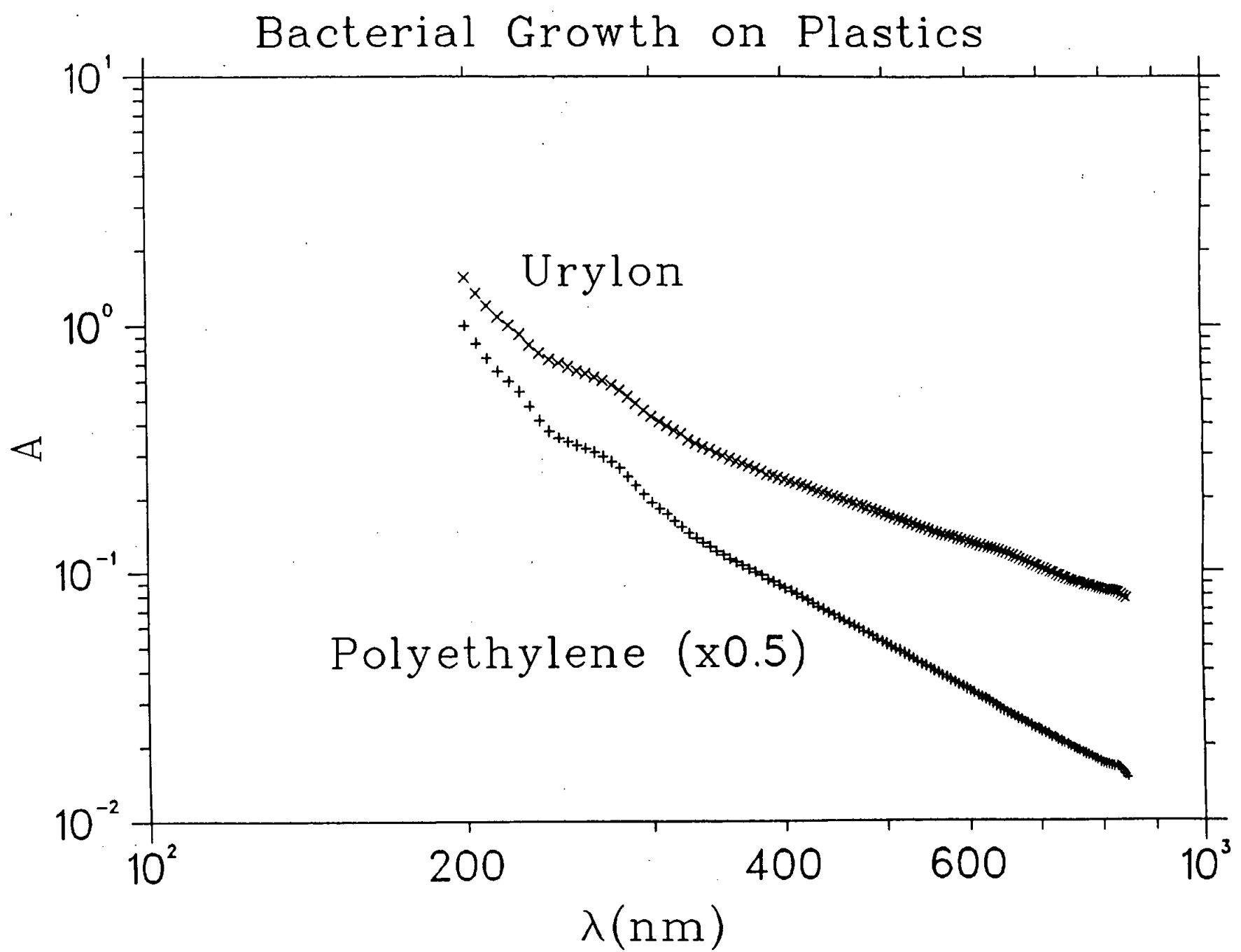
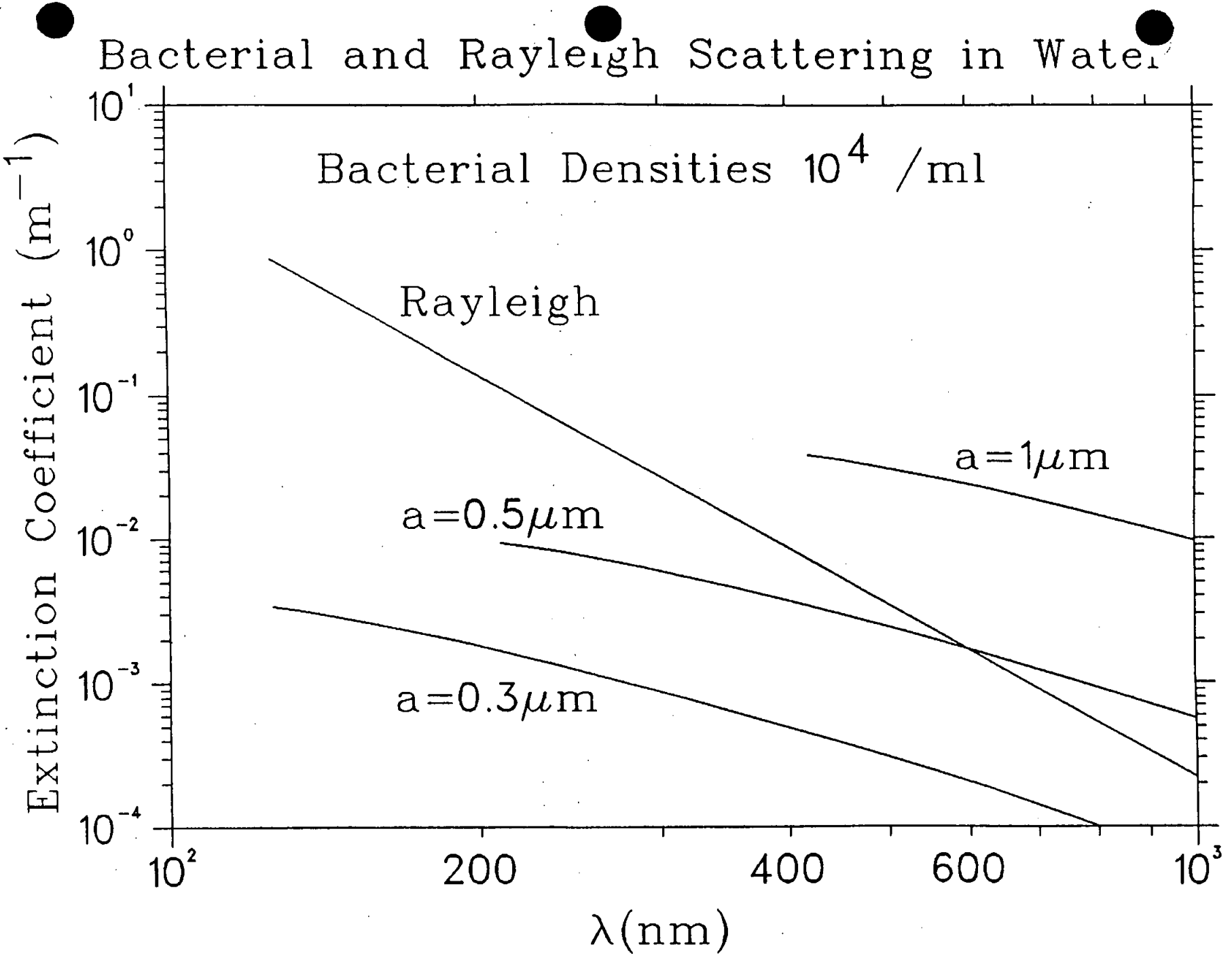

Power Law of Scattering from Small Spheres
$\sigma \alpha 1/\lambda^m$ at λ=600nm
m
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Sphere Radius (microns)

Scattering of E-coli in Variable-Index Media




Scattering + Absorption of E-coli
Points: data
Line: scattering theory
(5 x 10^7/ml)
Difference: absorption
A
.40
.35
.30
.25
.20
.15
.10
.05
.00
200
300
400
500
600
700
800
λ(nm)


Bacterial Growth on Plastics
Urylon
Polyethylene (x0.5)
A
λ(nm)
10^1
10^0
10^-1
10^-2
10^2
200
400
600
10^3


Bacterial and Rayleigh Scattering in Water
Bacterial Densities 10^4 /ml
Rayleigh
a=1μm
a=0.5μm
a=0.3μm
Extinction Coefficient (m^{-1})
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^2
200
400
600
10^3
λ(nm)