Impact of Particle Aggregated Microbes on UV Disinfection. II: Proper Absorbance Measurement for UV Fluence

Hadas Mamane[1] and Karl G. Linden, M.ASCE[2]

Abstract: Ultraviolet (UV) absorbance measurements are subject to significant error using a standard spectrophotometer when particles or aggregates that scatter light are present. True UV absorbance for highly turbid waters should be measured using integrating sphere (IS) spectrophotometry that allows the collection of reflected and transmitted radiation simultaneously. This is especially important when the effects of scattering impact UV disinfection—such as with the presence of aggregates. The impact of light scattering of particle-aggregated microbes on UV disinfection was evaluated by comparing standard spectrophotometer and integrating sphere absorbance measurements for UV fluence determination. Spore–clay aggregates in simulated drinking waters and spore aggregates with natural particles from raw waters were induced by flocculation with alum. Coagulated systems significantly decreased the UV inactivation effectiveness compared to the noncoagulated system with the effects more pronounced for raw natural water. Absorbance measurement of suspensions and aggregates using standard spectrophotometry in the calculations of fluence resulted in overdosing whereas the use of IS spectroscopy did not. The results demonstrated that aggregation protected spores from UV disinfection, and that use of proper absorbance measurement techniques, accounting for particle scattering, is essential for correct interpretation of the results.

DOI: 10.1061/(ASCE)0733-9372(2006)132:6(607)

CE Database subject headings: Reactors; Particles; Ultraviolet radiation; Disinfection; Microbes; Measurement.

Introduction

The impact of light scattering of microorganisms aggregated with particles on measurement of ultraviolet (UV) irradiation was studied. The use of UV irradiation for water disinfection purposes relies on accurate measurement of light absorption in water. Absorbance of natural waters is impacted by the various substances that affect its optical properties such as mineral particles, particulate organic matter, and microorganisms. The standard method used to measure light absorption relies on transmittance of light captured by a detector that is placed in front of the sample, using a single or dual beam spectrophotometer. The drawback of using the standard method relates to light scattering at angles outside the reception angle of the detector (Tassan and Allali 2002). Furthermore, with the standard spectrophotometer it is not possible to separate the incident beam from the light scattered in the forward direction, as the detector responds to a superposition of the incident and forward scattered fields (Bohren and Huffman 1983).

The fraction of light absorbed is generally obtained as the fraction of light transmitted subtracted from unity. However with particles that scatter light, the fraction of light absorbed (by the particles) equals the sum of the fraction of light transmitted and the fraction of light reflected all subtracted from unity (Hanssen 2001; Tassan and Allali 2002). Therefore measurement of UV absorbance for a suspension with large scattering can result in significant error in absorbance readings using a standard spectrophotometer (Tassan and Ferrari 2003). True UV absorbance, including all transmitted and scattered radiation, can be measured using integrating sphere (IS) spectrophotometry (Nelson and Prezelin 1993; Linden and Darby 1998, Tassan and Ferrari 2003). Integrating spheres are optical devices that integrate radiant flux (Castiglioni and Albertini 2000) as the geometry allows the collection of most reflected and transmitted radiation simultaneously, and presents an integrated signal or radiance field to the detector. The inside of the sphere is coated with a white thermoplastic material named Spectralon (Labsphere Inc., North Sutton, N.H.) that reflects over the entire UV-vis wavelength range (Storm and Springsteen 1998). Positioning the water sample inside the integrating sphere allows absorbance measurements of turbid samples in one measurement (Storm et al. 1998).

The average germicidal UV irradiance in water is a function of several factors including the irradiance (mW/cm^2) incident to the water, absorbance, and the pathlength of light in the water (Bolton and Linden 2003). The UV fluence (mJ/cm^2) is obtained by multiplication of the average germicidal irradiance by exposure time. Therefore there is a direct correlation between UV absorbance and the fluence a microorganism is exposed to. Consequently, not using integrating sphere spectroscopy to account for scattering in absorbance measurements will result in overdosing the UV system. For example, absorbance measurements using standard spectrophotometry were falsely higher compared to integrating sphere spectroscopy measurements for water suspensions with turbidity values above 3 NTU, which resulted in significant overdosing of bench scale samples (Christensen and Linden 2003).

[1]Research Associate, Porter School of Environmental Studies and School of Mechanical Engineering, Tel-Aviv Univ., Tel-Aviv, Israel 69978.

[2]Associate Professor, Dept. of Civil and Environmental Engineering, Duke Univ., P.O. Box 90287, Durham, NC 27708-0287 (corresponding author). E-mail: kglinden@duke.edu

Note. Discussion open until November 1, 2006. Separate discussions must be submitted for individual papers. To extend the closing date by one month, a written request must be filed with the ASCE Managing Editor. The manuscript for this paper was submitted for review and possible publication on August 13, 2004; approved on October 12, 2005. This paper is part of the ***Journal of Environmental Engineering***, Vol. 132, No. 6, June 1, 2006. ©ASCE, ISSN 0733-9372/2006/6-607–615/ $25.00.

Table 1. Experimental Design Used for Coagulation Experiments

Trial number	Coagulant dose (ppm alum)	Initial pH value	Spore concentration (cfu/mL)	Clay or particle turbidity (NTU)	Particle count of spore–particle suspension
1	0	5, 6.8, 8.2	3×10^4	0[b]	1.4×10^5
2	0	5, 6.8, 8.2	3×10^4	5[b]	9.2×10^7
3	0, 80	5	3×10^4	10[b]	1.7×10^8
4	0, 20	7.3[a]	3×10^4	15.8[c]	3.6×10^6
5	0, 20	7.3[a]	3×10^4	6.3[c]	2.4×10^6

[a]Natural water, no pH adjustment, the turbidity is of natural particles.

[b]Clay concentration.

[c]Particle concentration.

The impact of spore–clay suspension and spore–clay aggregates on UV inactivation of spores was previously tested (Mamane and Linden 2006). UV disinfection of spores in spore–clay aggregates (coagulant added) suspended in a simulated "natural" water resulted in lower log inactivation compared to spore–clay nonaggregated suspensions (no coagulant added). Ultimately, UV disinfection is negatively affected by aggregation that may occur in natural waters or during flocculation, if these flocs are not adequately removed. Previous research studies found that standard spectrophotometers overestimated the absorbance (Qualls et al. 1983) or the UV fluence (Linden and Darby 1998; Christensen and Linden 2003). However these studies investigated the effect of suspended particles on absorbance, and did not extend the study to include the effect of light scattering of particles cosuspended with microorganisms on the inactivation of the microorganism. Research on microbe–particle aggregation (Sobsey et al. 1991; Parker and Darby 1995; Emerick et al. 1999, 2000; Jolis et al. 2001; Loge et al. 2001; Templeton et al. 2003) has not previously included the effect of aggregate scattering on fluence determination and on microbial survival within the aggregate. A comparative study of UV inactivation for a cosuspension of particles and microbes (not associated with each other) and a suspension of particles aggregated with microbes properly taking into consideration scattering of those particles in absorbance measurements, has not been well documented for drinking water treatment. The goal of this study was to relate the impact of light scattering properties of suspended montmorillonite clay and natural particles from surface waters on UV disinfection of *Bacillus subtilis* spores aggregated or cosuspended with those particles.

Materials and Methods

Two different water types were utilized in the experiments: (1) a simulated drinking water (DW) spiked with spores and clay particles; and (2) natural raw water from the Wilson water treatment plant in Durham, N.C. spiked with spores. *B. subtilis* spore preparation and enumeration, clay particle preparation, simulated water preparation, analytical measurements, and particle size analysis were described in Part I (Mamane and Linden 2006). Briefly, simulated DW water was mixed with *B. subtilis* spores at concentration 3×10^4 cfu/mL and clay particles with turbidities of approximately 0, 5, and 10 NTU of Na-rich Montmorillonite clay particles (Crook County, Wyo.). In the other experiments, two samples of natural raw waters with turbidities of 6.3 and 15.8 NTU were mixed with *B. subtilis* spores at a similar concentration of 3×10^4 cfu/mL. *B. subtilis* spores (*ATCC 6633*) were enumerated by the pour plate technique. The overall experimental design and conditions for simulated and natural waters is presented in Table 1.

Integrating Sphere Spectroscopy

Conventional absorbance measurements, termed "direct" or "*D*," were performed with a UV-vis dual beam spectrophotometer (Varian, Model Cary 100BIO, Victoria, Australia) without the IS attachment in place. True absorbance measurements accounting for scattering by particles, termed "IS," were performed with the same spectrophotometer equipped with 150 mm diameter IS attachment [Labsphere Diffuse Reflectance accessory (DRA)-CA-30] and a center mount sample holder used to position the sample inside the IS, with a reflectance factor of 0.973 at 254 nm. The turbid sample is placed in a 1 cm path length quartz cuvette with all four windows optically polished. The cuvette is fixed to a spring loaded holder that is hanged in the center of the sphere, connected to the top sphere cover. A diagram of the center mount integrating sphere attachment is provided by Storm et al. (1998) and in Christensen and Linden (2003).

Effect of Suspended Clay Particles

The UV fluence was determined for a cosuspension of spores with clay particles in simulated drinking water using the integrating sphere (Table 1, Trials 1 and 2). The experimental design was as follows:

1. 0 NTU clay (direct): no clay particles were added to the spore suspension and the UV fluence was determined with absorbance measured by standard spectrophotometer;
2. 5 NTU (direct): 5 NTU clay particles were added to the spore suspension and the UV fluence was determined with absorbance measured by standard spectrophotometer; and
3. 5 NTU (IS): 5 NTU clay particles were added to the spore suspension and the UV fluence was determined with absorbance measured using integrating sphere.

Identical experimental setups were evaluated with suspensions of spores and clay particles at pH levels of 5.0, 6.8, and 8.2.

Suspended and Aggregated Particles

The conditions used to study the impact of scattering on UV inactivation of *B. subtilis* spores cosuspended or aggregated with clay or natural particles at different turbidities are described in Table 1, Trials 3, 4, and 5. Preliminary jar test experiments were conducted to determine the ideal alum concentration needed to form suspended aggregates of either spores with 10 NTU clay particles in synthetic waters or spores with natural particles from

the raw waters with turbidity of 6.3 or 15.8 NTU. Coagulation and flocculation experiments were performed at bench scale with a standard jar test apparatus. Different quantities of freshly prepared alum were added to each jar (0–80 ppm alum), mixed at 100 rpm for 2 min, reduced to 30 rpm for 20 min, and then settled for 30 min. After settling, the supernatant was analyzed for turbidity and spore count. The ideal alum dose for coagulation experiments corresponded to the lowest turbidity values in the supernatant. Homogeneous suspended aggregates (at ideal alum concentration) and noncoagulated suspensions were produced by mixing without settling in samples as follows:

1. sus *D* and sus IS represent suspended disperse system (without alum addition) with fluence determined using direct or IS absorbance values, respectively; and
2. agg *D* and agg IS represent suspended aggregated system (at ideal alum addition) with fluence determined using direct or IS absorbance values, respectively.

It is important to maintain floc stability during sampling and transport of aggregated systems. Even the sampling of flocs can cause disaggregation of these fragile structures (Droppo and Ongley 1992), therefore there is a possibility that aggregates disaggregated to a certain extent under exposure to UV due to mixing and under the particle size analysis procedure due to passage of particles through apertures.

Advanced Microscopic Techniques: Transmitted and Confocal Microscopy

Spore cells were observed using phase contrast microscopy (400X, Nikon E600). A nondestructive method was used to view the spore cells diluted in water by placing a drop of suspension on a microscope slide without staining or manipulating the sample. Spore aggregates were observed by additional microscopic methods such as differential interference contrast (DIC), polarized, and confocal. Transmitted imaging for DIC was obtained with a confocal laser scanning microscope (CLSM) (LSM 5 Zeiss Pascal). Polarized images were obtained by transmitted light polarization configuration with the polarizer set to east–west and the analyzer set to north–south (Olympus FV 500). Confocal imaging in reflection was obtained by CLSM (Olympus FV 500).

Low-Pressure UV Irradiation System and Data Presentation

Low-pressure UV irradiation system, fluence determination, and data presentation are described in Part I (Mamane and Linden 2006). Briefly, a bench scale UV apparatus consisted of low-pressure mercury vapor germicidal lamps emitting nearly monochromatic UV radiation at 253.7 nm directed through a circular opening provided incident UV radiation. The measured incident irradiance at the surface of the test liquid was corrected for nonhomogeneity of irradiation across the surface area of the Petri dish to provide the average incident irradiance, as follows:

$$E_{\text{avg}} = E_{\text{inc}} \frac{1 - e^{-[A \cdot 2.303 \cdot b]}}{A \cdot 2.303 \cdot b} \tag{1}$$

where E_{avg}=average UV irradiance (mW/cm^2); E_{inc}=incident UV irradiance (mW/cm^2); A=decadic UV absorbance of water sample measured at 254 nm (1/cm); and b=depth of water sample (cm).

UV fluence was calculated from the average UV irradiance according to the following equation:

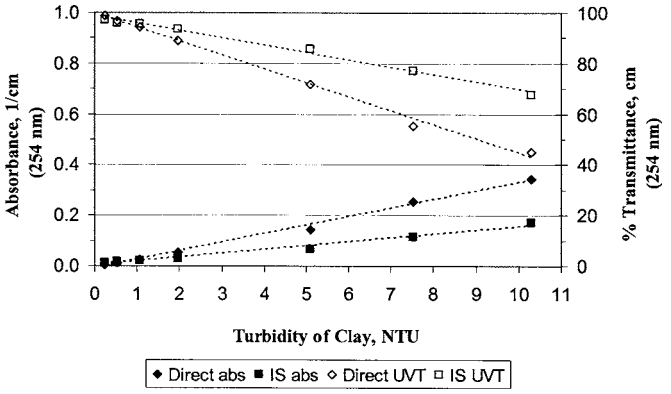


Fig. 1. Comparison of direct and integrating sphere absorbance and transmittance measurements at 254 nm of suspended clay particles as function of turbidity. Absorbance and transmittance are measured in 1 cm cuvette.

$$H = t \cdot E_{\text{avg}} \tag{2}$$

where t=UV exposure time (s); and H=UV fluence (mJ/cm^2).

Thus, exposure times were calculated by dividing the desired UV fluence by the average UV irradiance. Ten mL volumes of sample placed in 60 mm diameter sterile irradiation vessels were irradiated. Mean concentration [colony forming units/milliliter (cfu/mL)] of spores spiked in suspension without UV exposure was taken as the initial concentration, N_0. Duplicates of 10 mL samples of spore–water suspension were UV irradiated. Each duplicate was serially diluted twice and plated in triplicate per UV exposure. The mean concentration of spores after exposure (N_d) and standard deviation were summarized. The $\log_{10}$ transformation for N_0/N_d was plotted as a function of the UV fluence (H). Regression analysis was performed using all data points to fit the linear sections of the log inactivation curve, by the following equation:

$$\log_{10}\left(\frac{N_0}{N_d}\right) = k \cdot H \tag{3}$$

where k=fluence-based inactivation rate coefficient determined for each experimental run.

Results and Discussion

Absorbance Measurements of Suspended Clay Particles

Absorbance measurements of clay particles are performed by the conventional spectrophotometer and compared to absorbance as obtained by using the integrating sphere attachment. To collect all or most of the light scattered from samples it is important to mount the sample directly onto the center of the integrating sphere (Hanssen 2001) as was performed in these experiments. Fig. 1 illustrates the effect of UV absorbance or transmittance (at 254 nm, 1 cm cuvette) conducted by mounting an integrated spheres setup versus using a conventional spectrophotometer. Above values of 2 NTU clay particles, a difference between absorbance measurements is observable with falsely higher values of absorbance occurring with the use of the standard spectrophotometer. This difference in absorbance measurements increases with increasing turbidity of the water. Multiple scattering and

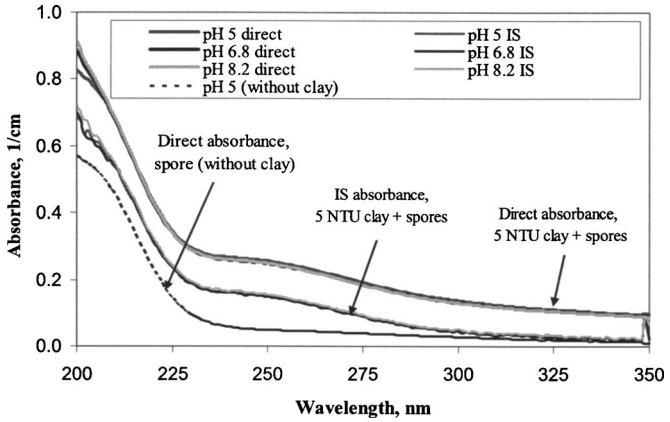


Fig. 2. Effect of pH on absorbance measurements of 5 NTU clay suspension in simulated waters. Absorbance is measured in 1 cm cuvette.

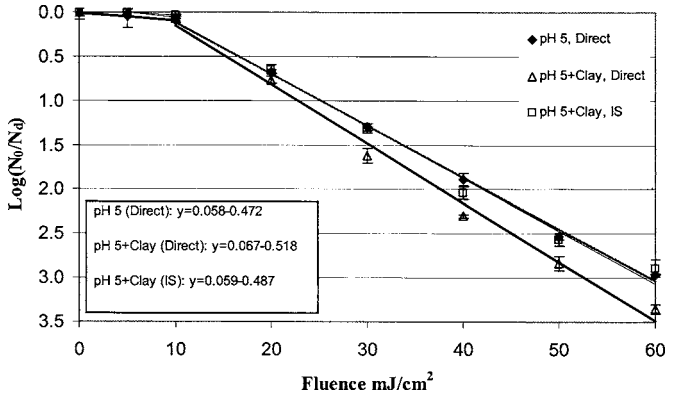


Fig. 3. Impact of 5 NTU suspended clay particles on UV fluence–response curves of spores, using direct and IS absorbance measurements for fluence determination. Error bars represent ±standard deviation (SD).

interparticle interferences are of concern for highly concentrated particle samples and the interparticle interferences can become significant in the case of highly charged particles or particles with refractive indices close to 1 (Schnablegger and Glatter 1995).

When calculating the average irradiance using Eq. (1), the direct absorbance measurement resulted in an underestimate of the average irradiance by 5% for all simulated waters with 5 NTU clay and spore particles. This value compares well with the 3.5% underestimation determined by Christensen and Linden (2003), with differences probably due to the nature of the particles used.

Fig. 2 illustrates the impact of pH of spores cosuspended with 5 NTU clay particles in simulated waters on direct and IS spectral absorbance. The difference between direct and IS values are similar throughout the whole wavelength range, thus in this case pH had no effect on absorbance or light scattering of clay particles. Absorbance measurements of water suspended with spores (without clay particles) demonstrate that the clay particles are the cause for scattering in simulated waters, as spores at the concentration used for this research did not scatter light.

Inactivation Response Using Direct and IS Absorbance

A difference in absorbance as measured by direct (D) and IS spectroscopy, when used to calculate delivered fluence, may result in a difference in the UV inactivation of spores. Fig. 3 illustrates the differences in log inactivation of spores with absorbance measured by D or by the IS method for suspended spores alone and for spores suspended with 5 NTU clay particles (without alum; Table 1, Trials 1 and 2). The fluence–response of spores suspended with clay particles as measured by D versus the IS technique differs substantially. Initially a lag in inactivation exists at a low UV fluence up to 10 mJ/cm^2. The difference in inactivation of dispersed spores or those cosuspended with clay particles increased with an apparent UV fluence from 10 to 60 mJ/cm^2, starting at 0.1 log difference at 20 mJ/cm^2 up to 0.5 log difference at 60 mJ/cm^2. Fluence determined with the direct absorbance measurement is conservative as it provides additional inactivation compared to the IS technique at the same apparent fluence. Thus, because of the falsely high absorbance value in the presence of particles by the direct measurement technique, the apparent delivered fluence is higher than it should be (if the true IS absorbance were measured) when clay particles at 5 NTU are suspended with spores.

The first order inactivation rate constant (indicated by the slope) for 0 NTU clay (direct) and 5 NTU clay (direct) are 0.058 and 0.067 cm^2/mJ, respectively. The goal was to test if the shoulder and the first order regression lines of dispersed spores and spores cosuspended with clay particles measured by the direct technique (Fig. 3) are represented statistically by the same or similar lines. Specifically it is important to assess whether similar slopes also have the same intercept, which indicates that the differences in the shoulder are also insignificant. The different combinations between shoulder and slopes tested for statistical significance are as follows: (1) both treatments (0 and 5 NTU, direct) with same slopes and intercepts; (2) both treatments with same slopes however with different intercepts; and (3) treatments are with different slopes and intercepts. The analysis of covariance (ANCOVA) method can be used to capture the interaction of the first order regression slope with a main effect (Sall et al. 2001) such as UV fluence with each treatment. Here, this method is accomplished by introducing the interaction between log inactivation of spores and UV fluence with each treatment, which is the main effect. The P value for the interaction is statistically significant at fluence levels of 10–60 mJ/cm^2 [$F(1,35)=18.8564$; $P=0.0001$] indicating that the difference between the inactivation rate constants for the direct measurement of spores in the absence or presence of clay particles is highly significant, therefore the slopes are different for these treatments.

Interestingly, the fluence response of suspended spores (without clay) as measured by direct technique, 0 NTU clay (direct), compared to the (true) fluence response of spores cosuspended with clay particles as measured by the IS technique, 5 NTU (IS) are not statistically significant. The ANCOVA model showed that the P value for the interaction is not significant at fluence levels of 10–60 mJ/cm^2 [$F(1,35)=0.1270$; $P=0.7239$] indicating that there is no difference between the inactivation rate constants for the fluence calculated using direct measurement of spores in the absence of clay particles and IS measurements of spores with clay particles. This finding provides statistical evidence that light scattering by particles is correctly taken into consideration when using the IS technique for fluence determination of scattering dispersed clay particles.

Table 2 provides a summary of the direct and IS UV fluence-based inactivation coefficients for suspended disperse spores and for spores cosuspended with clay, at different pH values. At all pH values the differences in the fluence-based inactivation

Table 2. UV Fluence-Based Inactivation Coefficient of Suspended Spores and Spores Suspended with 5 NTU Clay

pH	Spore, direct	Spore+clay, direct	Spore+clay, IS
5	0.058±0.001	0.067±0.002	0.059±0.002
6.8	0.060±0.001	0.068±0.002	0.060±0.001
8.2	0.058±0.002	0.065±0.003	0.058±0.001

Note: IS=integrating sphere. ± standard error for coefficient. Average irradiance determined using either direct or IS absorbance measurements. Coefficients of suspended spores as measured by direct technique versus spores cosuspended with clay particles as measured by the IS technique are similar. Coefficients of spores suspended with clay particles as measured by the direct technique versus the IS technique are different.

coefficients of suspended spores as measured by direct technique and spores cosuspended with clay particles as measured by the IS technique are not statistically significant. Moreover the difference in fluence-based inactivation coefficient of spores suspended with clay particles as measured by the direct technique versus the IS technique is statistically significant.

Impact of Particles on Absorbance and UV Inactivation of Spores

The ideal dose for aggregation of simulated waters spiked with spores (pH 5, turbidity of 10 NTU) occurred at 80 ppm alum, while the ideal dose for aggregation for natural raw waters (pH 7.3, turbidity of ~16 or 6.3 NTU) spiked with spores occurred at 20 ppm alum. The impact of clay or natural particles suspended or aggregated with spores at optimum alum concentration on absorbance, and UV inactivation at 40 mJ/cm^2, determined using direct and IS absorbance, is illustrated in Figs. 4–6. As previously shown in Part I (Mamane and Linden 2006) aggregated spores are protected from UV compared to nonaggregated spores and the difference in the spore counts between aggregated and dispersed systems indicates the significance of shielding on particle-associated spores. Aggregation of spores spiked into raw waters with natural particles occurred at 20 ppm alum compared to

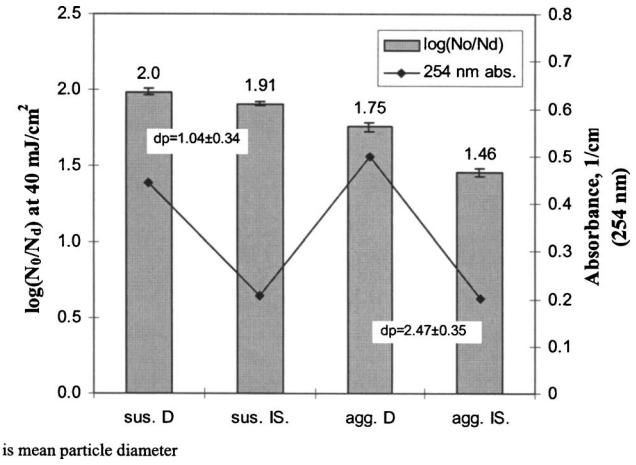


Fig. 4. Absorbance and UV inactivation for aggregated and suspended spores and clay in simulated water at 10 NTU turbidity. Ideal aggregation occurred at alum dose of 80 ppm. Error bars represent ±standard deviation. Absorbance is measured in 1 cm cuvette.

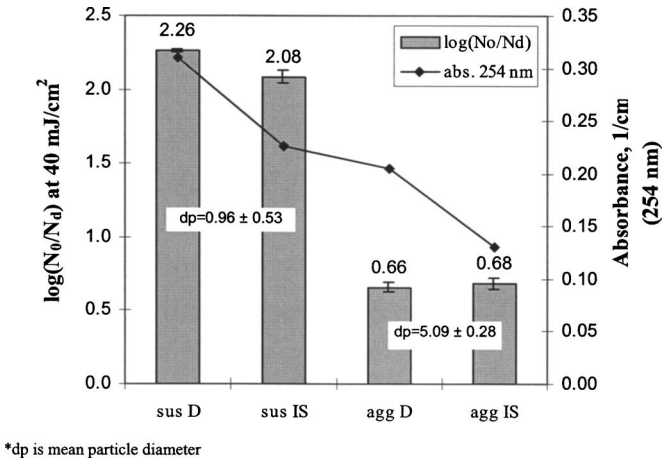


Fig. 5. Absorbance and UV inactivation of aggregated and suspended spores in natural water at 15.8 NTU turbidity. Ideal aggregation occurred at alum dose of 20 ppm. The error bars represent ±standard deviation. Absorbance is measured in 1 cm cuvette.

80 ppm alum of simulated waters, likely due to high alkalinity and phosphate of simulated waters and negative charges and particle size of clay in simulated waters.

Results illustrated in Figs. 4–6 show that the absorbance measurements are higher using direct compared to IS spectroscopy both for aggregates and suspended spores. In Fig. 4 the difference in inactivation at 40 mJ/cm^2 for both clay–spore aggregates and clay–spore suspensions indicate that fluence calculation using direct absorbance values results in increases of 0.1 and 0.3 log inactivation for the suspended and aggregated systems, respectively. Inactivation of spores (without clay) suspended in simulated DW as measured by direct absorbance is 1.9 log at 40 mJ/cm^2. Inactivation of spores suspended with 5 NTU clay particles as measured by direct absorbance is 2.3 log (Fig. 3) and with 10 NTU clay particles is 2 log (Fig. 4). With direct absorbance, an increased spore inactivation is expected when suspended with 10 NTU turbidity compared to 5 NTU due to the

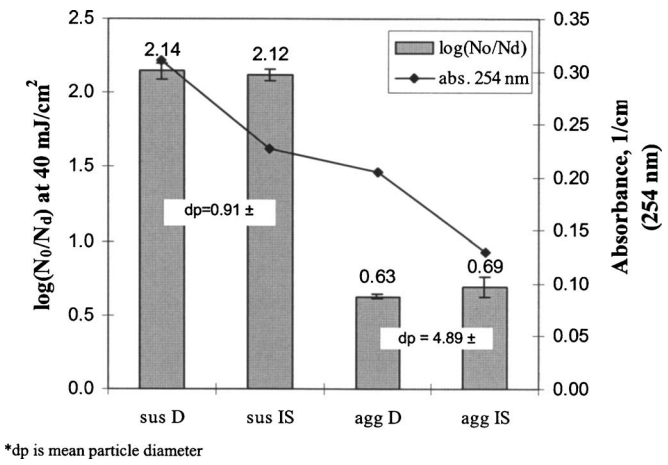


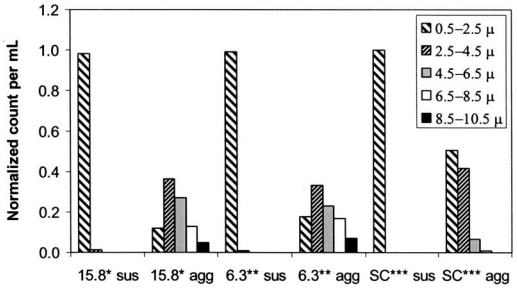
Fig. 6. Absorbance and UV inactivation of aggregated and suspended spores in natural water at 6.3 NTU turbidity. Ideal aggregation occurred at alum dose of 20 ppm. Error bars represent ±standard deviation. Absorbance is measured in 1 cm cuvette.

fluence overestimation that results in an increase of UV exposure time. Other factors such as multiple scattering and shielding probably affected the outcome as multiple scattering is usually apparent above 5 NTU (Huber and Frost 1998) and consequently scattering is probably not proportional to particle concentration. Use of absorbance measurements with IS of a suspension of spores with 5 and 10 NTU clay resulted in 1.9 log inactivation at 40 mJ/cm^2 (similar to inactivation of spore suspension by direct). Consequently, the same log inactivation of spores suspended with different turbidities was achieved, indicating that the integrating sphere corrects for particle scattering.

Inactivation of spores aggregated with 10 NTU clay particles in simulated DW as measured by D and IS at 40 mJ/cm^2 is 1.75 log and 1.46 log, respectively (Fig. 4). Inactivation of spores aggregated with 15.8 NTU of natural water turbidity as measured by D and IS absorbance, at 40 mJ/cm^2 is 0.66 log and 0.68 log, respectively (Fig. 5), while inactivation of spores aggregated with 6.3 NTU of natural water turbidity as measured by direct and IS absorbance is 0.63 log and 0.69 log, respectively (Fig. 6). In contrast to simulated waters, no difference in spore inactivation was apparent with aggregated systems using direct versus IS absorbance with natural waters. With the suspended systems, there was no difference in inactivation of spores at lower turbidity (6.3 NTU) however a nonsignificant difference (about 0.2 log) was evident with natural particles at higher turbidity values (15.8 NTU).

In both simulated and natural systems spores embedded within aggregates are apparently protected from UV light as indicated by lower inactivation compared to the suspended systems. In simulated water the difference in log inactivation between the suspended and aggregated system is 0.2–0.4 log inactivation, whereas this difference was 1.4–1.6 log in natural waters. Turbidity values of natural waters were higher than the maximum allowable drinking water turbidity (5 NTU) for unfiltered water supplies. At 6.3 NTU the difference between the suspended and aggregated system was 1.5 log, thus at the regulated limit of 5 NTU turbidity, inactivation will also likely be compromised if aggregation is present. Therefore, aggregates formed with natural particles were much more effective at "protecting" the spores from UV irradiation as compared to aggregates formed with clay particles.

The nature of particles as organic versus inorganic of natural and simulated waters, respectively, and the particle distribution may affect the extent of protection of spores in an aggregate. Natural particles vary in size and shape, while primary clay particles were less variable in size and shape, when excluding aggregates. Montmorillonite clay particles were chosen as representative of natural inorganic particles in water at the micron size range of typical colloids in water treatment plants. Particles from natural waters were evaluated due to the practical implications on UV disinfection of waters in a treatment plant. Natural environments consist of many particles that each scatter light differently and result in an integrated scattering field as measured by the integrating sphere. The total scattering field depends on the particle concentration in the sample (Bohren and Huffman 1983). Therefore not only the physical and chemical nature of the particle affects the scattered field, but also the particle concentration, which eventually impacts the UV fluence and the extent to which spores are "protected" from UV inactivation. High levels of scattering in aquatic particles are typical for samples consisting of inorganic mineral detritus particles (Tassan and Allali 2002). The inorganic portion of natural particles can be described by the ratio of the volatile to total suspended solids. Approximately 75–78% of surface water particles used in this study were comprised of inorganic material that theoretically should exhibit the higher scattering ability of inorganic particles. Developing correlations between water quality and scattering in natural waters may allow predictions of particle scattering effects on UV disinfection for different waters.



Note:
Sus – suspended non-aggregated system, agg – aggregated system at optimum alum dose.
* 15.8 represents spores suspended with 15.8 NTU natural particles or aggregated with 20 ppm alum.
* *6.3 represent spores suspended with 6.3 NTU natural particles or aggregated with 20 ppm alum.
*** SC represents spores + 10 NTU clay suspended in synthetic water or aggregated with 80 ppm alum.

Fig. 7. Particle counts for suspended and aggregated systems over 0–10 μm

Fig. 7 illustrates the relative particle count per milliliter of aggregates and suspensions divided into different size bands from 0.5–2.5, 2.5–4.5, 4.5–6.5, 6.5–8.5, and 8.5–10.5 μm. For all suspended particles the fraction between 0.5 and 2.5 μm is the most dominant fraction as spores and clay particles (in simulated waters) have a mean particle size of approximately 1 μm as described in Part I (Mamane and Linden 2006). For the aggregated system, dominant particle fraction is between 2.5 and 4.5 μm and between 0.5 and 2.5 μm for natural and simulated waters, respectively. The aggregated system shows a wider particle distribution compared to the suspended system. In addition, aggregates of natural waters contain particles in a larger size band (8.5–10.5 μm) compared to the spore–clay (SC) system in simulated DW with the largest size band between 4.5 and 6.5 μm. This difference likely has an effect on protection of spores in aggregates as the largest aggregates (in natural water) could protect spores more efficiently compared to smaller ones. Previous wastewater research indicates that the threshold particle size that bacteria associated with particles would be protected from UV irradiation was between 7 and 10 μm (Cairns et al. 1993; Emerick et al. 1999; Jolis et al. 2001). In wastewater, particle associated coliforms were greater than 10 μm and 12% of the total particles between 10 and 80 μm were associated with bacteria (Emerick et al. 2000). Based on the findings presented above, it is not immediately obvious which aggregate size is responsible for protecting spores. However it appears that in coagulated natural systems, aggregates above 6.5 μm are protective of spores. Each particle scatters light in different directions and patterns and therefore the mutual influence of scattering patterns from particles can lead to enhancement or cancellation of the integrated scattering effect. This effect will increase with particle size as more peaks and valleys in the scattering pattern will occur with larger particles. Shape is also important; if the particle is distorted, the scattering patterns are different (Bohren and Huffman 1983). Therefore the extent of scattering depends on geometrical factors such as scattering direction, size, and shape, in addition to particle chemical composition and concentration.

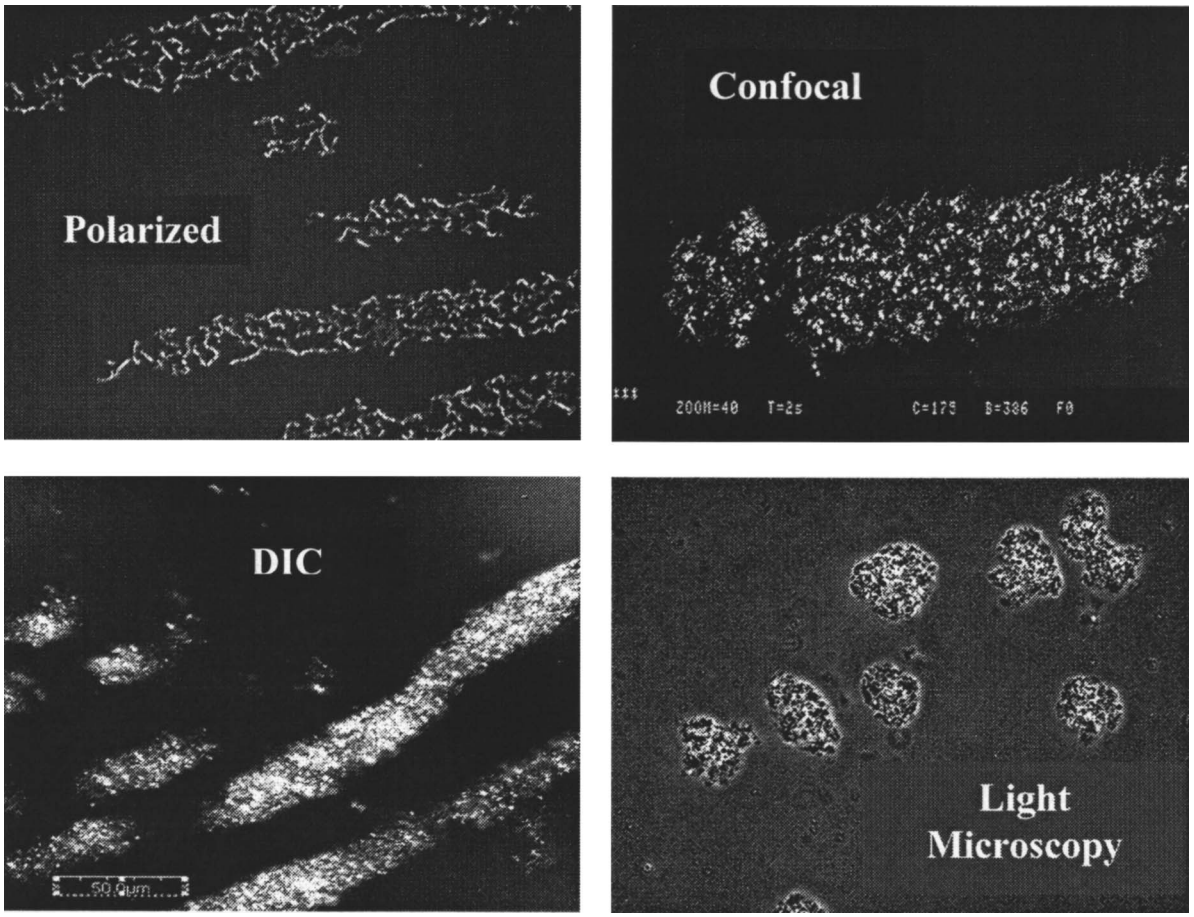


Fig. 8. Qualitative micrographs of spores aggregated with clay particles, using advanced microscopic techniques

Advanced Microscopy Techniques

Micrographs of spores aggregated with clay particles using advanced microscopic techniques are illustrated in Fig. 8. Spore–clay aggregates were observed by DIC, polarized, and confocal microscopy. All these techniques imaged the aggregate by simple preparation of placing a small drop of test water on a cover slip and attaching it to a microscopic slide to minimize disruption. The spores and the clay particles are highly reflective and therefore could be viewed under confocal microscopy. With all these images the flocs were with irregular shape and in various aggregate size. These images were viewed in order to study whether it is possible to use simple preparation techniques and various microscopes to view aggregates. The aggregates viewed were larger (even greater than 50 μm) than the average aggregate size obtained by particle size analysis. These results indicate that particle analysis and microscopic analysis of fragile structures such as aggregates are subject to methodological artifacts and may not be comparable.

The observed size of the aggregate measured both under numerous microscopy techniques and with the particle size analyzer raised the following questions: (1) what is the real aggregate size that is exposed to UV? (2) what is the real aggregate size that is efficient in protecting spores? and (3) what is the relationship between the dispersed and aggregated state of particles and microbes with relation to UV disinfection? These microscopy images are not highly informative for interpreting spore inactivation results, as these techniques inherently create artifacts. The observed microscopy images should be viewed in relation to the aggregation state of the microbe, and with regard to interpreting differences in inactivation kinetics. The effect of particle scattering on UV reactor dosimetry is likely related to diverse particle size, diverse refractive indices of particles and medium, particle concentration, the effect of particulate chemical characteristics, and wavelength dependency of scattering.

Practical Considerations for Water Treatment Plants

Water supplies are susceptible to spikes of particulates due to storm runoff, wastewater discharge to the water, winds that carry particulates to the water, or other events. In addition, waters may promote microbial interaction with particles that are surface associated or embedded within a particle aggregate. In the case of unfiltered waters, these particles may not be removed before disinfection. Particles or aggregates can absorb light or scatter light, which in the latter case will be available for disinfection but not measured by the UV detector. The typical sensors for UV transmittance measurements will measure decrease in ultraviolet transmittance (UVT) (increase in apparent absorbance) in the case of light scattering particles and may respond by setting an alarm for increasing the UV fluence to the system even when the reduction in transmittance of the water is apparent and not real. The outcomes from this research can be applied to actual practices of UV disinfection; however it is unlikely that water utilities would routinely employ sophisticated UV absorbance measurement and particle distribution analysis to try and carefully characterize the physical state of microorganisms and scattering ability of their

waters. Observations in this study can contribute to actual UV disinfection practices if water samples at the inlet of the UV reactor were periodically analyzed at specialized labs that have an integrating sphere to determine if scattering is an issue leading to UV overdosing. Another practical outcome can possibly relate to design of a sensor that directly measures or responds to scattered light and will thus result in calculation of the true fluence for UV reactors in the presence of scattering particles.

Light scattering of dispersed particles and aggregates will lead to "overdosing" the bulk water based on the apparent higher absorbance levels. Delivering an increased UV fluence is a potential advantage for public health protection and would provide a safety net in terms of disinfection, specifically because light scattering could indicate microbe protection due to the presence of particles. However it may be a disadvantage due to increases in the UV lamp power, which raises energy costs to the water utilities even when it is not really necessary. Unfiltered systems meeting the filtration avoidance criteria of the USEPA Surface Water Treatment Rule (SWTR) are not at any time allowed turbidities greater than 5 NTU prior to disinfection. Since unfiltered supplies do not have the barrier of filtration, the bias of the higher UV dose application may, in some instances, offset the potential risk of particles interfering with disinfection in these unfiltered supplies, however, if particles and microbes are strongly aggregated, this protection will not be overcome even by very high UV doses. In addition, to evaluate aggregation, various parameters should be historically measured, such as mean particle size and volume, particle size distribution (PSD), and filtered PSD. Further distinction into different size bands can help define the extent to which microbes may be protected in an aggregate. Thus, dividing particle counts into fraction of counts below and above certain values (such as 2–3 μm), will differentiate between dispersed and aggregated particles. Natural particles aggregated with microorganisms will require an increased UV fluence compared to a dispersed system of particles cosuspended with particles to achieve disinfection, although in some cases, there may be microbes that are protected even at elevated doses. As a result, analysis of particle size distribution with a particle analyzer will provide insights into the extent of particle aggregated microbes in a water sample that may occur naturally or as a result of filter breakthrough. Water utilities should consider that turbidity is not the most important parameter when evaluating the effect of particles on UV disinfection efficacy. Particles add turbidity to waters but also may associate with microorganisms in an aggregate. Turbidity and absorbance measurements of waters are not reliable indicators of aggregation because particle-associated microbes can exist with low turbidity, and low absorbance values in the bulk water.

Conclusions

1. For water with turbidity >3 NTU, the use of direct conventional absorbance measurement in the calculations of fluence leads to overdosing of the UV system.
2. The direct measurement underestimated the average bench scale irradiance by 5% for all synthetic waters cosuspended with 5 NTU clay particles and spores.
3. Use of IS spectroscopy can overcome the problem with conventional spectroscopy in absorbance measurements of scattering suspensions.
4. All spore–particle aggregates protected spores, however aggregates of natural particles in water protected spores to a greater extent compared to aggregates of added clay particles in simulated waters.
5. Coagulated aggregates of spores in natural water contained larger sized particles compared to the SC system, which indicates particle size may be an important measure to relate to particle–microbe aggregation and effects on disinfection.

Acknowledgments

The writers gratefully acknowledge Professor Regina Sommer, Institute of Hygiene, University of Vienna, Vienna, Austria, for providing *Bacillus subtilis* spores for this research, and Professor Robert Bagnell, the University of North Carolina at Chapel Hill, who provided microscopic facilities and advice for aggregate imaging. They also thank Professor Michael Lavine and Gil Bohrer, Duke University, Durham, N.C. for their assistance in statistical analysis. The Wilson Water Treatment Plant in Durham, N.C. supplied water used in this research. This research was funded by the USEPA Science to Achieve Results (STAR) Program, Grant No. R82-9012. At the time of this research, Dr. Mamane was a doctoral candidate in the Department of Civil and Environmental Engineering at Duke University.

Notation

The following symbols are used in this paper:

A = UV absorbance of water sample measured at 254 nm;
b = depth of water sample (cm);
E_{avg} = average UV irradiance (mW/cm^2);
E_{inc} = incident UV irradiance (mW/cm^2);
H = UV fluence (mJ/cm^2);
k = fluence-based inactivation rate coefficient determined; and
t = UV exposure time (s).

References

Bohren, C. F., and Huffman, D. R. (1983). *Absorption and scattering of light by small particles*, Wiley-Interscience, New York.

Bolton, J. R., and Linden, K. G. (2003). "Standardization of methods for fluence (UV dose) determination in bench scale UV experiments." *J. Environ. Eng.*, 129(3), 209–215.

Cairns, W., Sakamoto, G., Comair, C., and Gehr, R. (1993). "Assessing UV disinfection of a physico-chemical effluent by medium pressure lamps using a collimated beam and pilot plant." *Proc., Planning, Design & Operation of Effluent Disinfection Systems, Water Environment Federation Specialty Conf.*, WEF, Whippany, N.J.

Castiglioni, E., and Albertini, P. (2000). "An integrating sphere to measure CD from difficult samples." *Chirality*, 12(4), 291–294.

Christensen, J., and Linden, K. G. (2003). "How particles affect UV light in the UV disinfection of unfiltered drinking water." *J. Am. Water Works Assoc.*, 95(4), 179–189.

Droppo, I. G., and Ongley, E. D. (1992). "The state of suspended sediment in the freshwater fluvial environment. A method of analysis." *Water Res.*, 26(1), 65–72.

Emerick, R. W., Loge, F. J., Ginn, T., and Darby, J. L. (2000). "Modeling the inactivation of particle-associated coliform bacteria." *Water Environ. Res.*, 72(4), 432–438.

Emerick, R. W., Loge, F. J., Thompson, D., and Darby, J. L. (1999). "Factors influencing ultraviolet disinfection performance. II: Association of coliform bacteria with wastewater particles." *Water Environ.*

Res., 71(6), 1178–1187.
Hanssen, L. (2001). "Integrating-sphere system and method for absolute measurement of transmittance, reflectance, and absorptance of specular samples." *Appl. Opt.*, 40(19), 3196–3204.
Huber, E., and Frost, M. (1998). "Light scattering by small particles." *J. Water Supply: Resarch and Technology*, 47(2), 87–94.
Jolis, D., Lam, C., and Pitt, P. (2001). "Particle effects on ultraviolet disinfection of coliform bacteria in recycled water." *Water Environ. Res.*, 73(2), 233–236.
Linden, K. G., and Darby, J. L. (1998). "Ultraviolet disinfection of marginal effluents: Determining ultraviolet absorbance and subsequent estimation of ultraviolet intensity." *Water Environ. Res.*, 70(2), 214–223.
Loge, F. J., Bourgeous, K., Emerick, R. W., and Darby, J. L. (2001). "Variations in wastewater quality parameters influencing UV disinfection performance: Relative impact of filtration." *J. Environ. Eng.*, 127(9), 832–837.
Mamane, H., and Linden, K. G. (2006). "Impact of particle aggregated microbes on UV disinfection. I: Evaluation of spore-clay aggregates and suspended spores." *J. Environ. Eng.*, 132(6), 596–606.
Nelson, N. B., and Prezelin, B. B. (1993). "Calibration of an integrating sphere for determining the absorption-coefficient of scattering suspensions." *Appl. Opt.*, 32(33), 6710–6717.
Parker, J. A., and Darby, J. L. (1995). "Particle-associated coliform in secondary effluents: Shielding from ultraviolet light disinfection." *Water Environ. Res.*, 67(7), 1065–1075.
Qualls, R. G., Flynn, M. P., and Johnson, D. (1983). "The role of suspended particles in ultraviolet disinfection." *J. Water Pollut. Control Fed.*, 55(10), 1280–1285.
Sall, J., Lehman, A., and Creighton, L. (2001). "Fitting linear models." *JMP Start Statistics*, 2nd Ed., Duxbury Thompson Learning, Pacific Grove, Calif., 305–328.
Schnablegger, H., and Glatter, O. (1995) "Sizing of colloidal particles with light-scattering—Corrections for begining multiple scanning." *Appl. Opt.*, 34(18), 3489–3501.
Sobsey, M. D., Fuji, T., and Hall, R. W. (1991). "Inactivation of cell associated and dispersed Hepatitis-A virus in water." *J. Am. Water Works Assoc.*, 83(11), 64–67.
Storm, S. L., and Springsteen, A. (1998). "Choosing the right sphere size for your application—The integrating sphere reflectance accessory." ⟨http://www.labsphere.com/knowledge.asp⟩ (July 21, 2004).
Storm, S. L., Springsteen, A., and Ricker, T. M. (1998). "A discussion of sample mount center holder designs and applications—The use of center mount sample holders in reflectance spectroscopy." ⟨http://www.labsphere.com/knowledge.asp⟩ (July 21, 2004).
Tassan, S., and Allali, K. (2002). "Proposal for the simultaneous measurement of light absorption and backscattering by aquatic particulates." *J. Plankton Res.*, 24(5), 471–479.
Tassan, S., and Ferrari, G. M. (2003). "Variability of light absorption by aquatic particles in the near-infrared spectral region." *Appl. Opt.*, 42(24), 4802–4810.
Templeton, M. R., Andrews, R. C., Hofmann, R., and Whitby, G. E. (2003). "UV inactivation of floc-associated MS2 coliphage." *Proc., 76th Annual Water Environment Federation Technical Exhibition and Conf., (WEFTEC)*, WEF, Los Angeles.