Protocol for the Determination of Fluence (UV Dose) Using A Low-Pressure or Low-Pressure High-Output UV Lamp in Bench-Scale Collimated Beam Ultraviolet Experiments

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The following Protocol was adopted by the IUVA Board of Directors in April, 2015. The Board agreed that the Protocol should be published as a Draft Protocol and allow comments to be submitted over a period of one year. If you wish to submit a comment for suggested changes, please send it to the Technical Committee at editorinchief@iuva.org. Revisions, if any, that result from these comments will be presented to the IUVA Board in April 2016 for final endorsement.

INTRODUCTION

This Protocol is based on the paper by Bolton and Linden (2003), but it is set out in a step-by-step manner to make it easier to follow for experimental measurements. The reader should first read the Bolton and Linden (2003) paper to understand the background for this Protocol. Also, convenient Excel spreadsheets are available either from the authors or in the Member Zone of the web site of the International Ultraviolet Association (www.iuva.org). The users of this Protocol should also consult Appendix C of the USEPA Ultraviolet Disinfection Guidance Manual (UVDGM, 2006), which is largely based on the Bolton and Linden (2003) paper. This Protocol provides more detail than does the UVDGM. However, the UVDGM also provides an excellent analysis of uncertainties in collimated beam testing. At various points in this Protocol, reference will be made to UVDGM (2006) where this Protocol differs from or expands on the protocol in the UVDGM.

BACKGROUND

It is assumed that the user has available a quasi-collimated beam apparatus set up according to the instructions in Bolton and Linden (2003), a radiometer and detector calibrated within the past 12 months at 254 nm, a Petri dish to contain the suspension of microorganisms, a small Teflon® coated stir bar and a stirring motor mounted on a platform that can be adjusted vertically. This Protocol is specific for the use of low-pressure (LP) or low-pressure high-output (LPHO) mercury vapor lamp(s). A separate Protocol for medium-pressure (MP) lamps is being developed.

Figure 1 shows two examples of a suitable collimated beam apparatus.

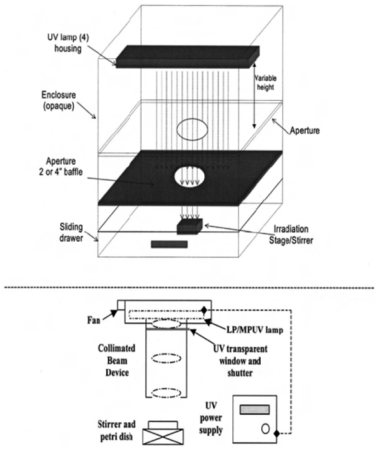

Figure 1. Examples of suitable collimated beam setups: (top) a setup using apertures to define the beam; (bottom) setup using a collimating tube to define the beam [taken from Bolton and Linden (2003)]

TERMS AND UNITS

Irradiance (symbol *E*; units W m^{-2}) is defined as the total radiant power *incident* from all directions in a hemispherical solid angle *on* an infinitesimal element of surface of area dS containing the point under consideration divided by dS.

Fluence Rate (symbol E_o; units W m^{-2}) is defined as the total radiant power incident from all directions through an infinitesimally small sphere of cross-sectional area dA, divided by dA.

Note that in a collimated beam the irradiance and the fluence rate are virtually the same.

The *fluence* (symbol *F*, units J m^{-2}) (also called UV dose) is the time integral of the fluence rate and is defined as the total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere of cross-sectional area dA, divided by dA. If the irradiance is constant in time, the fluence (UV dose) is given by $E \times t$, where t is the exposure time in seconds.

In North America, it is common to use the units mW cm^{-2} for fluence rate or irradiance and mJ cm^{-2} for fluence (UV dose). Note that 1 mW cm^{-2} = 10 W m^{-2} and 1 mJ cm^{-2} = 10 J m^{-2}.

PROCEDURE WHEN A LOW PRESSURE OR LOW PRESSURE HIGH OUTPUT UV LAMP IS USED

In this case, the UV output from the UV lamp is emitted almost exclusively at 253.7 nm (at least in the germicidal 200 – 300 nm range). Hence, it can be considered to be essentially a monochromatic UV source. In the Protocol below, reference is made to the Excel spreadsheet "Fluence - LP - (revised - 12-Dec-2014)_Final_for_IUVA Board.xlsx". It is assumed that the radiometer detector is not sensitive outside of the germicidally relevant wavelength range (such as with an International Light 240 SEL or SED detector, or with a NS254 interference filter).

Preliminary setup

- Choose a sample volume for the suspension of microorganisms and enter this volume into cell G31 in the *Fluence Calculations* worksheet of the Excel spreadsheet. This volume, plus the volume of the stir bar, is used to calculate the sample depth. Alternatively, the user may choose to measure the sample depth directly and put this value into cell G35.
- Enter the volume of the stir bar into cell G32 in the *Fluence Calculations* worksheet of the Excel spreadsheet. This volume can be determined from caliper measurements of the length and diameter of the stir bar or by displacement using at least 10 stir bars of equal size.

Determination of the incident irradiance

- It is most important to place the radiometer detector such that it reads the irradiance incident on the top surface of the water in the Petri dish. One method is to have a ruler mounted vertically beside the stirring motor platform so that the Petri dish can be raised, such that it is at the same vertical position as the calibration plane of the radiometer detector. Another method is to use a laser beam to define the vertical position of the top of the solution in the Petri dish. Enter the incident irradiance at the center of the Petri dish into cell G40 in the *Fluence Calculations* worksheet of the Excel spreadsheet.

- Alternatively, a sensor can be mounted beside the Petri dish to measure continuously the relative incident irradiance. By averaging this sensor reading over the time of the UV exposure, and by comparison with calibrated radiometer readings, the average absolute incident irradiance can be determined accurately.

- It is also important that the radiometer and detector have been calibrated by the radiometer manufacturer within a period of no more than 12 months before the date of the measurements using a NIST -traceable (or other equivalent national standards laboratory) calibration procedure. Calibration can be achieved either by returning the radiometer and detector to the manufacturer for recalibration or by using the KI/KIO_3 actinometer calibration Protocol (Bolton et al., 2011) [see separate IUVA Protocol (in preparation)]. Furthermore, the user should be aware that if the irradiance is too high, the detector will saturate. This information can be obtained from the radiometer manufacturer. Thus one should make sure that the irradiance level is well below the point where saturation sets in. Typical collimated beam experiments have an incident irradiance between 0.1 and 1 mW cm^{-2}. Note that the UVDGM (UVDGM, 2006, Section C2.2, page C5) requires the use of two radiometers and detectors. Recalibration is required only if the two instruments give readings that differ by more than 5%. Nevertheless, each radiometer must have been recalibrated within 12 months prior to the date of use.

Determination of the Petri Factor (PF)

- Determine the inner diameter (cm) of the Petri dish and enter it into cell G33 of the *Petri Factor* worksheet of the Excel spreadsheet.

- On a light-colored cardboard, draw two perpendicular lines that cross at the center of the cardboard. Draw ticks every 0.5 cm from the center intersection and label the ticks according to the distance from the center.

- Place the light-colored cardboard on the stir motor and the radiometer detector on top of the cardboard. Adjust the level so that the calibration plane of the radiometer detector is at the level that the top surface of the water in the Petri dish will be during exposure.

- Center the stir motor so that the center of the light-colored cardboard is at the center of the light circle projected by the collimated beam.

- Allow the UV lamp to warm up until the output stabilizes, typically for at least 10 min.

- Place the radiometer detector so that the center of the detector is at the center of the light-colored cardboard; record the irradiance and the position of the radiometer detector.

- Progressively move the detector in 0.5 cm steps in the $+x$, $-x$, $+y$, and $-y$ directions, recording the irradiance at each position. Go out far enough so that the distance is greater than the inner radius of the Petri dish.

- Enter the radiometer detector data into columns S and X of the Petri Factor worksheet of the Excel spreadsheet.

- Note the value of the Petri Factor in cell K34 or CW148. If the Petri Factor is less than 0.90, use a Petri dish with a smaller diameter or a larger diameter collimator. For best practice, the Petri Factor should not be less than 0.90.

Determination of the Water Factor (WF)

- Place a sample of the suspension of microorganisms into a quartz spectro¬photometer cell and determine the absorbance at 254 nm. If the absorbance in a cell with a 1 cm path length is less than 0.0458 (UVT > 90%), the UVDGM recommends use of a cell with a path length of at least 4 cm.

- The absorption coefficient a is given by A/l, where l is the absorption cell path length (cm). Enter a into cell G37 in the Fluence Calculations worksheet of the Excel spreadsheet.

- The Water Factor is displayed in cell K31.

Determination of the Divergence Factor (DF)

- Measure the distance (cm) from the top surface of the Petri dish to the axial centerline of the UV lamp (i.e., half the diameter of the lamp). Enter this value into cell G36 in the Fluence Calculations worksheet of the Excel spreadsheet.

Reflection Factor (RF)

- Assuming that water is the medium, the Reflection Factor for a wavelength of 254 nm is 0.975 (cell K33 in the Fluence Calculations worksheet of the Excel spreadsheet).

Determination of the average irradiance in the suspension of microorganisms

- The average irradiance E_{avg} in the suspension of microorganisms is given by

$$E_{avg} = E_{incident} \times PF \times WF \times DF \times RF$$

and is displayed in cell G43 in the *Fluence Calculations* worksheet of the Excel spreadsheet.

Determination of the exposure times for the target fluences (UV doses)

- Enter the target fluences (UV doses) in to cells E46 to E52 in the Fluence Calculations worksheet of the Excel spreadsheet.

- The exposure times for the target fluences (UV doses) are the given in cells I46 to J52 in the Fluence Calculations worksheet of the Excel spreadsheet.

- Note that ideally, the exposure times should be at least 30 seconds to minimize any time measurement errors. If the calculated exposure times are less than 30 seconds, it is suggested to move the platform to a greater distance from the UV lamp to decrease the irradiance. The Petri factor will also need to be re-determined for this new distance.

Determination of fluence (UV dose) – Response Curves

The following is an example of a suggested protocol for determining fluence (UV dose) response curves. Experienced users will use best practice and standard operating procedures to establish their own protocol.

- Prepare a set of culture plates sufficient in number to handle the exposures planned.

- Choose a set (normally at least 6 including zero) of target fluences (UV doses) that cover an inactivation range of at least 4 logs. For example, the set might be 0, 15, 30, 45, 60, 75 mJ cm^{-2}. The actual fluence values will depend on the UV sensitivity of the specific microorganism. If collimated beam testing is being used for the purposes of UV reactor validation, it is important to consider that extrapolation of the dose response curve outside of the measured range of UV dose is not recommended per section C.3 of the UVDGM. Thus, in the development of the collimated beam test plan, it is recommended that the maximum UV dose be selected to provide a log inactivation that is slightly greater than the maximum log inactivation expected for the biodosimetry testing conducted on the same day as the collimated beam sample is collected.

- Randomize the set. In a study where triplicate determinations are conducted, a possible sequence of exposures and corresponding target fluences (UV doses) could be 60, 15, 75, 45, 0, 30, 75, 30, 0, 15, 45, 60, 15, 45, 0, 30, 75, 60. If only duplicate measurements are conducted, use 12 of the above target UV doses. Randomization assures that any systematic errors are converted to random errors.

- In accordance with UVDGM (2006, Section C.2.3, page C6), the incident irradiance should be re-measured with the calibrated radiometer and detector before and after each sample exposure. The irradiance value used to calculate UV dose should be the average of the pre- and post-exposure radiometer readings. If a second radiometer is utilized, in accordance with UVDGM (2006, Section C.2.2, page C-4), at a minimum, the second radiometer should be used to determine the measurement accuracy of the CB radiometer at least at the beginning and the end of each collimated beam test session.

- The '0' samples in the above set should be placed into position in the collimated beam apparatus and left there stirring for a certain time, but with the shutter closed. If three '0' samples are used, one should be for the longest exposure time used, one for the shortest and one a middle time.

The assay of any microorganisms should be performed by a qualified micro¬biologist. The steps below are general guidelines and suggestions learned from experience in carrying out collimated beam testing, and refer to culture analysis methods. Cell culture analyses will have different steps, but detailed micro¬biological procedures are beyond the scope of this protocol.

- UV exposed samples should be diluted 10-fold according to the expected viable cell count levels. At least two dilutions that bracket the expected count levels should be used.

- Assay at least three culture plates with each diluted suspension. Maintain a sterile environment during the assays.

- Culture the plates for the specified time at the specified temperature for the microorganism used, and then count the colonies or plaques on each plate.

- Follow best practices for determining the plates with countable colonies/plaques or plaques based on the size of the culture plate and the type of organism assayed. For instance, this may be those plates with between 20 and 200 colonies. For each set of plates that falls within the countable range, average the counts and, using the dilution factor, determine the viable concentration in the original suspension.

- While it is typical to use only one dilution range with countable colonies, it is also acceptable to calculate the viable count using a weighted average across different dilutions that are countable (Standard Methods, 2012).

- Determine the average log inactivation for each test condition from $\log[N_0/N]$, where N is the viable concentration for a given fluence (UV dose) and N_0 is the average of the viable counts for zero UV dose. Alternatively, one can use the y-intercept of a regression line calculated from the experimental data points (recommended by the UVDGM Protocol, Section C.3, page C-8, Step 1).

- The Fluence (UV dose) – Response Curve is a plot of $\log[N_0/N]$ on the Y-axis as a function of the fluence (UV dose) on the X-axis. An example is shown in Figure 2. Alternatively, the UV dose can be plotted on the Y-axis versus the $\log(N_0/N)$ values on the X-axis as is recommended by the UVDGM Protocol.

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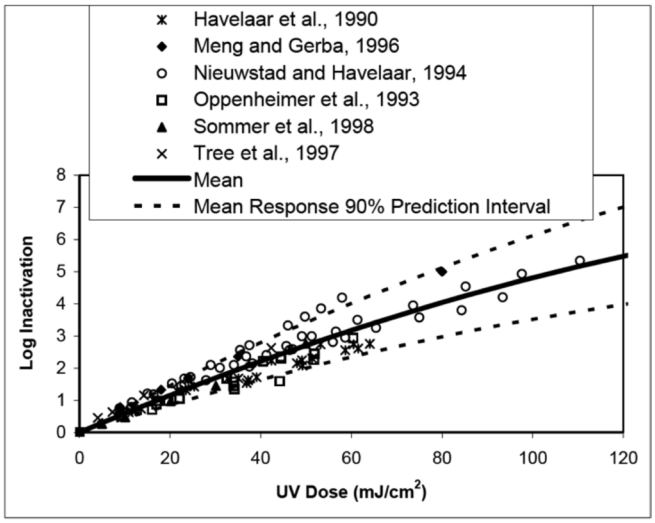

Figure 2. An example of a fluence-response curve, from the USEPA UV Disinfection Guidance Manual (UVDGM, 2006). Figure A.1. UV-Dose response of MS2 phage.

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