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1 The Use of Biodosimetry to Measure the UV-C Dose Delivered to a Sphere, and Implications
2 for the Commercial Treatment of Fruit.

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6
7 Abstract

8 Commercialization of UV-C treatment of horticultural produce in order to induce beneficial
9 responses in the produce following treatment requires both accurate dose delivery and a method
10 of treating large quantities of produce efficiently. Furthermore, it has long been assumed that
11 such effects require the entire surface of the horticultural commodities - typically fruit - to be
12 exposed to UV-C. This has invariably been achieved by manually rotating the fruit in a UV-C
13 field whilst reducing the dose delivered at each rotation in direct proportion to the number of
14 rotations. However, the resulting UV-C dose distributions achieved under these circumstances
15 are generally not reported in the literature. In the work described here a polystyrene sphere (Dia.,
16 70 mm) was used to simulate fruits such as tomatoes, apples, peaches etc., that have an
17 approximately spherical form in order to provide a means of measuring the total doses of UV-C
18 accumulated during treatment and comparing such estimates to theoretically-derived ones. This
19 was achieved using dosimetry based on spores of *B. subtilis* in which spore-impregnated
20 membranes were attached to the surface of the sphere. The fraction of spores surviving exposure
21 was used to estimate dose from a dose-response curve for the spores. Under irradiation
22 conditions leading to a theoretically calculated dose of 10.6 J, spore dosimetry yielded estimates
23 of 9.1, 10.7 and 6.1 J for UV-C delivered in respectively, one, two or four exposures. In the case
24 of exposure of the sphere during continuous mechanical rotation for the same length of time (80

25 s) a value of only 3.5 J was obtained. Irradiation conditions resulting in the spores being subject
26 to intermittent exposure to UV-C led to dose estimates below the theoretically derived ones.
27 The circumstances under which spore dosimetry can be used to obtain surface dose distributions
28 are discussed.

29

30 Keywords: UV-C Hormesis, UV-C Dose Measurement, *Bacillus subtilis* spores, Biodosimetry,
31 Commercial UV Processing.

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45 1. Introduction

46 The proportion of post harvest losses of fruits and vegetables has been conservatively estimated
47 as being of the order of 20 % in developed countries (Wilson and Wisniewski, 1989; Dal Bello et
48 al., 2008) and as high as 50% in developing countries (Wilson and Wisniewski, 1989). Whilst
49 these estimates conceal geographic, crop-specific and other variations, such levels of losses can
50 no longer be accepted as inevitable, or indeed sustainable, when viewed in terms of the scarce
51 resources needlessly consumed at a time when the world's demand for food is failing to be met.

52 One proposal that is attracting attention for increasing the shelf life of horticultural
53 commodities, and hence reducing food wastage, is the application of low doses of shortwave
54 ultraviolet light (UV-C). This form of treatment has been referred to as 'hormetic' i.e. providing
55 beneficial outcome from an agent (UV-C in this case) that at high doses can prove detrimental
56 (Calabrese and Blain, 2009). This type of application needs to be differentiated from the more
57 conventional application of UV-C conducted to directly inactivate micro-organisms present at,
58 or near, the surface of the horticultural commodities. Hormetic treatment is intended to result in
59 the induction of anti-microbial plant metabolites that occurs over a period of time *following* the
60 application of UV-C treatment (Shama, 1999). The potential that hormetic UV-C treatment
61 holds for the horticultural sector has recently been reviewed (Shama and Alderson, 2005), and
62 one benefit in particular is that decreased reliance would be placed on exogenously applied
63 chemical agents such as fungicides (Escalona et al., 2010).

64 Optimal UV-C doses are typically obtained as a result of experimental studies conducted at a
65 small scale and often, by the treatment of individual commodities –typically fruit. Because, as
66 inferred above, UV-C has the potential to damage plant tissue at sufficiently high doses, it is
67 important to be able to accurately deliver doses that have been experimentally found to elicit
68 hormetic effects. Such considerations would be crucial in commercializing UV-C treatment
69 (Shama, 2007). However, it is first necessary to investigate whether the modes by which fruit

70 have been treated in previous experimental studies are all equivalent. In the majority of previous
71 studies workers have attempted to ensure that the entire surface of the fruit receives exposure to
72 UV-C by manually rotating the fruit 2 or 4 times. In such cases the dose delivered to each 'side'
73 of the fruit is reduced in direct proportion to the number of times it is rotated (Stevens et al.,
74 2005; Charles et al., 2008; Yang et al., 2009). However, in none of these studies were surface
75 dose distributions experimentally determined.

76 One method of achieving this is through the application of spore dosimetry (Tyrrell, 1978). In
77 this method the dose-response behaviour to UV-C of microbial spores is first obtained and then
78 the fractional survival of spores is determined under conditions where it is desired to estimate
79 the UV-C dose. Doses may then be computed from the dose-response curve. Spores of *Bacillus*
80 *subtilis* have frequently been used for this purpose owing to the fact that they are not pathogenic
81 (Gardner and Shama, 1999). Spore dosimetry itself comes under the general category of
82 'biodosimetry', i.e. measuring the response of a biological agent to the effects of electromagnetic
83 radiation. In the work described here we examine whether spore dosimetry can be used to
84 estimate the doses of UV-C delivered to the surface of a polystyrene sphere under conditions of
85 exposure designed to emulate those mentioned above that have been used in laboratory studies
86 with fruit. Manual rotation of fruit would obviously not constitute a viable commercial method
87 of treatment, and therefore we extended our investigation to include one method (mechanical
88 rotation) that could potentially enable different types of produce to be irradiated with UV-C
89 ensuring both that consistent doses are achieved and that the dose distribution is relatively even
90 over the surface of the produce. In all cases the integrated UV-C dose was estimated by attaching
91 membranes onto which spores of *B. subtilis* had been deposited at various points on the surface
92 of the sphere, and these are compared with computed estimates of doses.

93

94 **2. Materials and methods**

95 2.1 UV Apparatus

96 The apparatus used for irradiating polystyrene spheres with UV is shown in Figure 1. The UV
97 source used was a low pressure mercury burner (GX018TSL, Voltarc Tubes Inc., Fairfield, CT.,
98 USA) having principal emission at 253.7 nm and rated at 42 W. This source was located within a
99 parabolic reflector fabricated from anodised aluminium. Immediately below the source was a
100 roller assembly driven by a variable speed electric motor (not shown). The entire source-reflector
101 assembly could be raised or lowered above the rollers to change the UV-C intensity. For static
102 treatment of polystyrene spheres, the spheres were placed on the cylindrical rollers but with the
103 motor turned off. For irradiation of membranes impregnated with spores (see below), the roller
104 assembly was removed and the membranes were treated on a stainless steel plate placed centrally
105 below the source. The intensity at the membrane surface was measured using a radiometer
106 (Model UVX, UV Products Ltd., Cambridge, Cambs.).

107

108 2.2 Preparation of Dose-Response Curve

109 Spores of *B. subtilis* (ATCC 6633) were produced according to the method described by Gardner
110 and Shama (1999) and stored at 4°C until needed. Spore suspension (1 mL) was filtered through
111 a 13 mm dia. Durapore® membrane with a retention of 0.22 µm (Millipore (UK) Ltd., Watford,
112 Herts) and then dried for 5 minutes in a laminar flow hood. This procedure was highly
113 consistent and resulted in the deposition of from 3.0 to 3.2 x 10⁶ spores per membrane. After
114 treatment, spores were recovered by placing the membrane in tubes containing 1 mL Ringer's
115 Solution and 5 glass ballotini beads (4 mm) and agitated using a vibratory mixer for 5 minutes
116 and the spore suspensions thus obtained were serially diluted as necessary. Aliquots (100 µL)
117 were then plated onto the surface of Tryptone Soya Agar (Oxoid Ltd., Basingstoke, Hants). The
118 plates were then incubated at 30°C overnight and then counted. All experiments were conducted

119 in duplicate. Plots were then made of the log of reduction in spore viability ($\log N/N_0$) against
120 delivered dose to give the Dose-Response Calibration Curve for *B. subtilis*.

121 *2.3 Preparation and Irradiation of Polystyrene Spheres*

122 Shallow indentations (0.5 mm deep) were made in the surface of polystyrene spheres (dia. 70
123 mm; Fred Aldous Ltd., Manchester, Lancs.) using a stainless steel rod of 15 mm dia. This
124 enabled the membranes prepared as described above to be securely attached to the surface of the
125 spheres. The membranes were further secured in place by 50 μm thick discs of UV-C transparent
126 perfluoroalkoxy (PFA) film (Polyflon Technology Ltd., Stone, Staffs) held in place by narrow
127 strips of double-sided adhesive tape. Imagining the 'north pole' of a sphere to represent 0° ,
128 membranes were placed at 0, 45, 90, 135 and 180° (Figure 2a). For static treatment the spheres
129 were irradiated as follows; a) irradiation for 80 seconds b) irradiation for 40 sec. after which the
130 sphere was rotated through 180° before receiving a further irradiation of 40 sec. c) irradiation for
131 20 seconds followed by three rotations of 90° at which irradiation was for 20 seconds at each
132 rotation.

133 For treatment under rotation, spheres were treated singly for either 80 or 160 seconds at the
134 same intensity at a rotational speed of 10 rpm. In a further series of experiments spheres were
135 treated as above but with identical 'blank' spheres either side of the test sphere. These spheres
136 did not contain spore-laden membranes at their surfaces but were introduced to establish
137 whether their presence would reduce the amount of UV-C energy incident on the test sphere.

138 *2.4 Estimating the Total UV-C Dose Delivered to Spheres by Measuring Spore Survival*

139 Figure 2b depicts a sphere within a UV-C field; if a spherical segment has an area dA then the
140 energy falling on the surface of the segment is given by:

$$141 \quad dE = D(y)dA \quad (1)$$

142 where $D(y)$ is a function denoting the variation of UV-C dose at the surface. Substituting the
143 area of a segment of thickness dy into equation (1) gives:

$$144 \quad dE = D(y) * 2\pi y(x) \sqrt{dx^2 + dy^2} \quad (2)$$

145 Because the object in the UV-C field is a sphere, the function $x(y)$ may readily be computed. The
146 total UV-C energy falling on the sphere is obtained by integrating (1):

$$147 \quad E = 2\pi r \int_0^{2r} D(y) dx \quad (3)$$

148 In the work conducted here the dose was determined using spore dosimetry at points 1-5. $D(y)$
149 was obtained by fitting a polynomial function to the experimental points.

150 *2.5 Theoretical Estimation of the Total UV-C Dose Delivered to Spheres*

151 Knowledge of the UV-C intensity at any point on the sphere enables the intensity at any other
152 point to be calculated using the inverse square law:

$$I_2 = I_1 \left(\frac{y_1^2}{y_2^2} \right) \cos \theta$$

153 Where I_1 is the intensity at distance y_1 from the UV-C source and I_2 is the intensity at distance y_2
154 from the source and θ is the orientation of a tangent drawn at the surface of the segment with
155 the x-axis.

156 In the work reported here the sphere was divided into 5 segments and I_1 (3.1 mW/cm^2) was
157 measured using a UV-C radiometer.

158 *2.6 Statistical Analysis*

159 Analysis of variance (ANOVA) was carried out using commercially available software
160 (SIGMAPLOT 11; Systat Software Inc., San Jose, USA) on all experimental determinations of
161 delivered UV-C doses.

162 **3. Results and discussion**

163 The dose response curve for spores of *B. subtilis* is shown in Figure 3. Using this figure the
164 measured log reductions in spore viability were ‘translated’ into UV-C doses expressed as
165 mJ/cm².

166 Table 1 depicts the reductions in spore viability at each position of the sphere at which
167 membranes were attached along with the corresponding UV-C dose estimates. The values shown
168 represent the means from two separate experiments. For the case of a single exposure for 80 s,
169 the highest dose recorded (178 mJ/cm²) was at position 1. The dose at position 3 is only 10 % of
170 that at position 1, whilst at positions 4 and 5 no reduction in spore viability was detected
171 implying a zero dose.

172 Delivering the UV-C dose in 2 exposures each of 40 s resulted in doses at positions 1 and 5 of
173 92.0 mJ/cm², that is, 52 % of that for a single exposure. Where the dose was delivered in 4
174 consecutive exposures each of 20 s duration with rotation through 90 ° after each exposure, the
175 doses at positions 1, 3 and 5 ranged from 49.2 to 58.2 mJ/cm², which represented 30 % of the
176 value for a single exposure. Using the methods described above in Materials and Methods the
177 total, or integrated, UV-C dose delivered to spheres were calculated from experimental
178 measurements and also from theoretical considerations and are displayed in Table 2. Although
179 based on five experimental point readings of dose, the geometric symmetry of the test object (a
180 perfect sphere) enabled these predictions to be made with confidence. The theoretically-derived
181 doses are all equal to 10.6 J, however, the dose distribution is markedly different for each case
182 and is depicted in Figure 4. As expected, rotation of the sphere in the UV-C field four times
183 results in the most even dose distribution.

184 Good agreement with the theoretically-derived total dose is obtained from the spore dosimetry
185 experiments when the sphere was irradiated either once or twice (Table 2). However, for the case

186 of four rotations the method employed here gave a total dose of only 6.1 J - considerably below
187 the calculated value. The errors shown alongside the doses were computed using the polynomial
188 used to fit the data in the dose response curve (Figure 3) and from estimates of the errors in
189 determining the reductions in spore viability. For the former cases (no rotation of the sphere, or
190 only one rotation) each of the spore-laden membranes received only a single exposure to the
191 UV-C source, however, for four rotations each of the membranes would have received two
192 exposures of correspondingly reduced doses of UV-C with a short time interval between each
193 exposure.

194 In experiments conducted using the mechanical rollers it was observed that although the weight
195 of the polystyrene spheres (c. 5.6 g) was considerably lower than that of typical fruit of the same
196 diameter – an orange, for example, would weigh approximately 200 g – at the speed of rotation
197 employed here the spheres did not display a tendency to roll or spiral in a lateral direction. Under
198 these conditions of irradiation the total apparent dose for 80 s exposure was only 3.5 J. This was
199 the same time of exposure used for the spheres that were manually rotated and is only 33 % of
200 the theoretical dose. Doubling the exposure to 160 s gave an increased dose of 10.2 J – close to
201 the values obtained above. In order to establish whether this form of irradiation employing
202 rollers could form the basis of a practical, commercially-based process for treating produce, the
203 effect of interference from adjacent spheres was evaluated. To do this a sphere with spore-laden
204 membranes attached to it was placed on the rollers and on either side of it were placed blank
205 spheres – i.e. without membranes. A reduction in spore inactivation was observed at positions 1
206 and 5 (Table 3), that is along the axis of rotation, but the total dose delivered was 8.9 J which
207 represents only a relatively small reduction compared to the case above for a single sphere.

208 The case of the sphere given 4 exposures to UV-C and the spheres rotated on the rollers are
209 similar in that the spores located on the membranes were subject to, in the first case, as pointed
210 out above, 2 exposures to the UV-C source separated by a short time interval, and in the latter

211 case multiple exposures separated by somewhat shorter time intervals. The effects of intermittent
212 exposure to UV-C on microbial inactivation have previously been studied. Harm (1980) found
213 that survival in such instances was greater than if the dose were delivered in a single exposure.
214 This was attributed to the operation of DNA repair mechanisms during those intervals when the
215 microbial cells were not actually exposed to UV-C. Significantly, spores of *B. subtilis* are known
216 to possess the facility for repairing UV-C induced damage (Slieman and Nicholson, 2000).

217 This phenomenon constitutes in effect a limitation to the application of spore dosimetry for UV-
218 C dose determination. For cases where spores would receive only a single exposure to UV-C the
219 results presented here show that the method should prove useful and readily applicable. Spore
220 biodosimetry could be used to obtain estimates of dose distribution on the surface of objects of
221 irregular geometry or in cases where an object receives irradiation by more than one UV source
222 where mathematical predictions would become complex. However, limitations could arise if the
223 conditions of dose delivery result in an interval between UV-C dose accumulation at the surface
224 of an object. Apart from the roller device described here, this could arise if the object were being
225 conveyed in a UV tunnel with a discrete number of sources resulting in intervals of time when
226 the surface of the object were not being irradiated (Shama, 1999).

227

228 It has become the convention in experimental studies to cite UV-C doses in terms of energy
229 delivered per unit area – e.g. J/m^2 (Shama and Alderson, 2005) rather than in terms of *total* UV-C
230 dose delivered. The former are obtained by multiplying the UV-C intensity by the time of
231 exposure. The reluctance to give total doses stems from the fact that whilst it is possible to
232 calculate the total dose delivered for objects of regular geometry, horticultural produce rarely
233 conforms to this mathematical convenience. Notwithstanding, certain fruits such as apples,
234 tomatoes, citrus fruit and peaches could be considered to a first approximation as perfect
235 spheres. Calculating the total UV-C dose delivered to a head of broccoli would prove more

236 challenging, whilst calculating the dose delivered to a bunch of grapes would require a
237 considerably greater mathematical effort. Irrespective of this, the methods described here should
238 permit delivered doses to be measured when objects of irregular geometry – i.e. fruits and
239 vegetables – are exposed to sources of UV-C.

240

241 The issue of whether it is even necessary to irradiate the entire surface of horticultural products
242 is one that requires consideration. Mercier et al. (2000), attempting to prevent *Botrytis cinerea*
243 infection of carrots, found that UV-C did not have a systemic effect, and that it was necessary to
244 ensure full surface exposure. Moreover, these workers showed that resistance to infection was
245 closely associated with the accumulation in the carrot tissue of 6-methoxymellein which only
246 accumulated where the tissue had received direct irradiation. In such cases it would be useful to
247 have surface dose distribution plots such as are shown in Figure 4 in order to ensure that the
248 threshold UV-C dose for eliciting the plant response was being achieved over the entire surface.
249 On the other hand, Stevens et al., (2005) showed that for apples, peaches and tangerines the
250 greatest resistance to a variety of mould-induced rots were obtained by delivery of the UV-C
251 dose at the stem end of the fruit without rotation. It may turn out that whether or not full
252 surface exposure to UV-C is necessary may be dependent on the type of produce and it is
253 evident that further studies are required to determine this.

254

255

256

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259 Engineering, Loughborough University for useful discussion.

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301

302 **Figure Captions**

303 Figure 1 Schematic of UV Equipment

304 Figure 2 Polystyrene Sphere used in Experimental Studies

305 Figure 2a Location of Spore-laden Membranes

306 Figure 2b Estimation of the Total UV Dose Delivered to a Sphere

307 Figure 3 UV-C Dose Response Curve for Spores of *Bacillus subtilis*

308 Figure 4 Theoretically-Derived UV-C Dose Distributions for Spheres under Different

309 Conditions of Exposure

310 a Single Exposure (The UV-C Dose was delivered in one exposure of 80 s)

311 b Two Exposures (The sphere was irradiated for 40 s, rotated through 180 ° and irradiated for a further 40 s)

312 c Four Exposures (The sphere was irradiated for 20 s then rotated through 90 °; this was repeated a further 3
313 times).

314

315

Position		Mode of Exposure to UV-C (number of exposures x time at each exposure)					
		1 X 80 s		2 X 40 s		4 X 20 s	
		log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)
Number ¹	Angle (Degrees)						
1	0	-1.5	178.1 _a ±3.2	-1.22	92.0 _a ±7.5	-0.96	54.5 _a ±0.1
2	45	-1.4	129.1 _b ±5.2	-1.10	73.8 _b ±0.1	-0.64	27.2 _b ±3.2
3	90	-0.4	18.7 _c ±0.5	-0.60	24.9 _c ±2.7	-0.92	49.2 _a ±5.4
4	135	0.0	0.0 _d	-1.13	83.2 _b ±9.8	-0.66	27.2 _b ±2.7
5	180	0.0	0.0 _d	-1.22	92.0 _a ±7.5	-1.00	58.2 _a ±3.6

317

318 Table 1: UV Doses² Delivered to a Sphere (Dia., 70 mm) following Different Modes of
 319 Exposure as Estimated from *B. subtilis* spore dosimetry

320 1 Refer to Figure 2

321 2 Average of two readings with standard deviation

322 Within the same column dose values bearing different subscripted letters are significantly different ($P \leq 0.05$)

323

Number of Rotations in the UV Field	UV Dose (J)	
	Experimental ¹	Theoretical
Single	9.1 a \pm 0.9	10.6
Two	10.7 a \pm 1.0	10.6
Four	6.1 b \pm 0.6	10.6

Table 2: Comparison of Total UV-C Doses Delivered to Spheres under Different Conditions of Exposure as Determined by *B. subtilis* Spore Dosimetry and by Calculation.

'Single exposure' denotes that the sphere was irradiated by the UV-C source for 80s; 'Two Exposures' that the sphere was irradiated for 40 s, rotated through 180 ° and irradiated for a further 40 s; 'Four exposures' that the sphere was irradiated for 20 s and rotated through 90 ° and that this was repeated a further 3 times.

¹ Experimentally determined UV dose values with percentage errors from dose response plot (Figure 3)

324 Within the same column dose values bearing different subscripted letters are significantly different ($P \leq 0.05$)

325

Conditions and Time of Exposure		Single Sphere (80 Seconds)		Single Sphere (160 Seconds)		Sphere with Neighbour (160 Seconds)	
Position	Angle (Degrees)	log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)
1	90	-0.47	18.6 _a ±1.5	-0.79	35.6 _a ±1.0	-0.39	15.5 _a ±0.6
2	45	-0.65	27.7 _b ±0.6	-0.85	45.9 _b ±5.7	-0.82	39.5 _b ±4.5
3	0	-0.91	49.2 _c ±0.2	-1.39	136.9 _c ± 2.6	-1.34	135.9 _c ±1.6
4	45	-0.66	27.7 _b ±0.6	-0.85	45.9 _b ±5.7	-0.82	39.5 _b ±4.5
5	90	-0.49	18.6 _a ±1.5	-0.79	35.6 _a ±1.0	-0.42	17.2 _d ±1.0

Table 3: UV-C Doses¹ Delivered to a Sphere Rotated at 10 rpm under Different Conditions.

The notation “Single Sphere” indicates that only one sphere was present on the roller assembly during treatment, whereas “Sphere with Neighbours” denotes that blank spheres were placed either side of the test sphere.

1. Average of two readings with standard deviations

Within the same column dose values bearing different subscripted letters are significantly different ($P \leq 0.05$)

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327

328

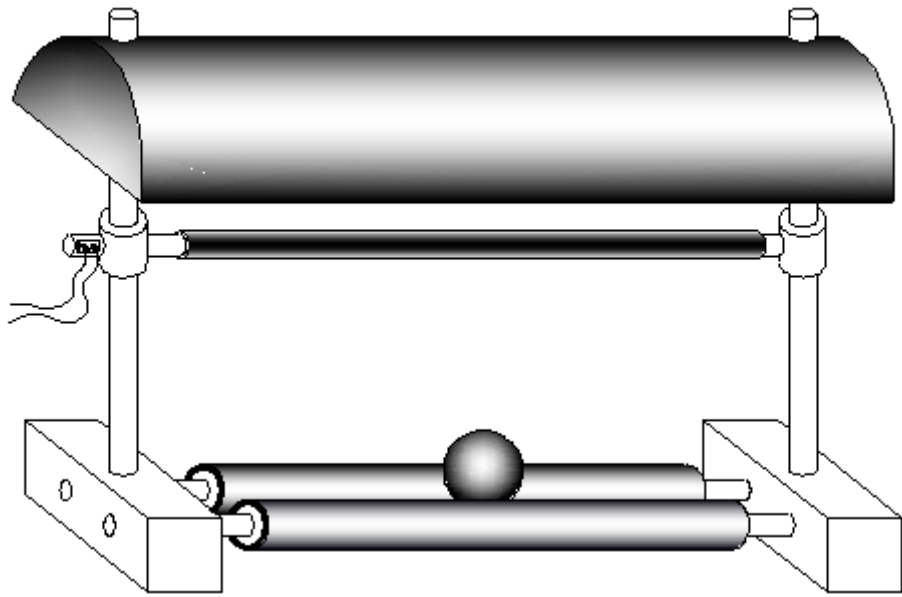


Figure 1

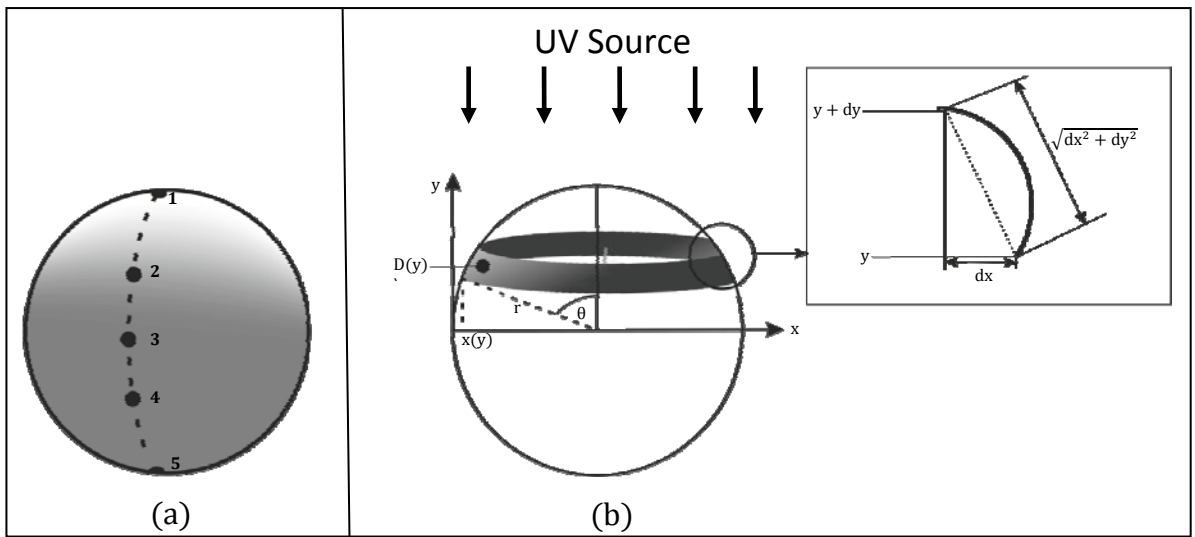


Figure 2

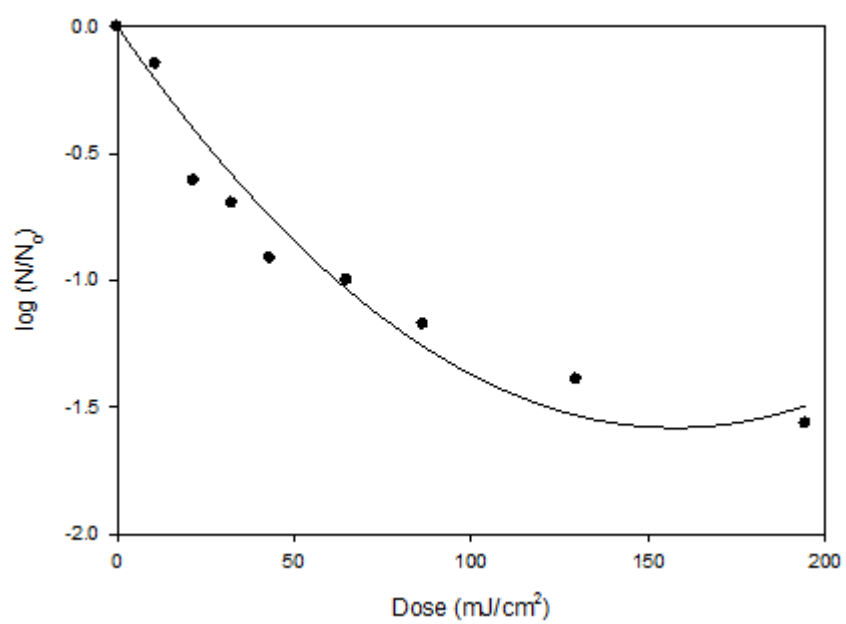


Figure 3

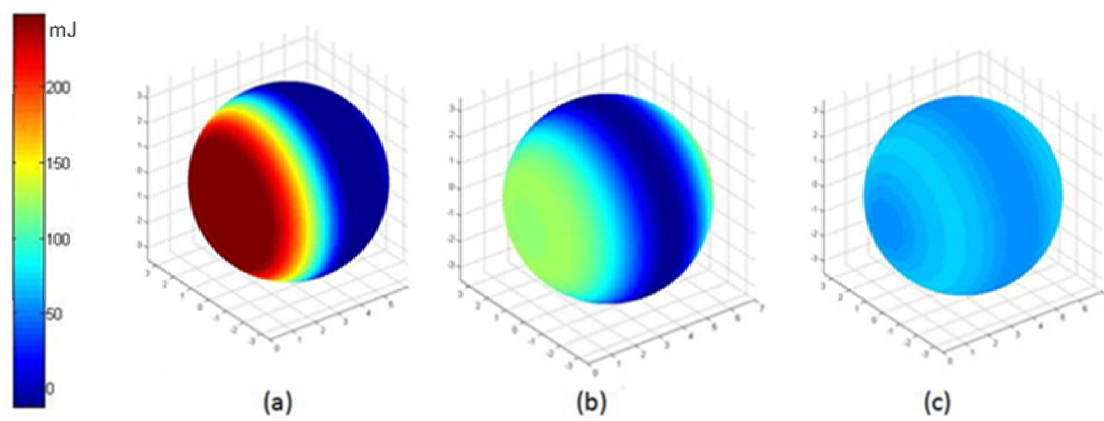


Figure 4

1