Protocol for Quantification of the Hydroxyl Radical Scavenging Term Draft

1. Hydroxyl Radical Scavenging Term

Advanced oxidation processes (AOP) generate hydroxyl radicals ($HO^{\bullet}$) which react with both the trace contaminants (≤ µg/L level) and several of the major solutes (> 1mg/L) typically found in natural waters. The major solutes that compete with the target contaminants for the generated $HO^{\bullet}$, thereby reducing the AOP treatment efficiency, are termed $HO^{\bullet}$ scavengers. Typically, these include bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and a complex mixture consisting of dissolved organic matter that is measured and expressed as dissolved organic carbon (DOC) (Stumm and Morgan 1996). The aggregate of these scavengers exerts an $HO^{\bullet}$ scavenging demand represented by a scavenging term (ST) that may be expressed as the summation of the products of their respective second order $HO^{\bullet}$ reaction rate constants, $k_{HO,S}$, and concentrations $[S_i]$ as shown in Equation (1).

$$\sum k_{HO,S_i} \cdot [S_i] = k_{HO,S_1} \cdot [S_1] + k_{HO,S_2} \cdot [S_2] + k_{HO,S_3} \cdot [S_3] + \cdots \quad (1)$$

The ST is a *pseudo*-first order rate constant for $HO^{\bullet}$ decay through the reactions with the water matrix constituents and has units of s^{-1}. The value of ST will be site specific, largely due to the unknown composition and reactivity of the DOC with $HO^{\bullet}$. While the magnitude of ST is difficult to predict for a given water, it will impact the sizing and operation of AOP equipment. It is therefore necessary to derive the magnitude of ST by calculating it experimentally.

2. Principle of Analytical Methodology

An indirect determination of ST may be obtained by the addition of the probe compound, e.g., parachlorobenzoic acid (pCBA), and hydrogen peroxide (H_2O_2) to a sample of the water to be tested, followed by irradiation of the mixed sample using a bench-scale collimated beam apparatus equipped with a low-pressure mercury vapor lamp, measurement of the degradation kinetics of the probe compound, and computation of ST as shown in Equation (2).

$$\mathrm{ST} = \sum k_{HO,S_i}[S_i] = \frac{\ln(10) \cdot \epsilon_{H2O2} \cdot \Phi_{HO} \cdot [H_2O_2] \cdot k_{HO,C}}{U_{253.7} \cdot k'_{HO}} \quad (2)$$

Where:

ϵ_{H2O2} is the molar absorption coefficient of H_2O_2 (L mol^{-1} cm^{-1});

Φ_{HO} is the overall quantum yield of $HO^{\bullet}$ generation from H_2O_2 at 253.7 nm (dimensionless);

$[H_2O_2]$ is the initial concentration of H_2O_2 in solution (mol L^{-1});

$k_{HO,C}$ is the second order hydroxyl radical reaction rate constant of probe compound (L mol^{-1} s^{-1});

$U_{253.7}$ is the energy per mole of photons at 253.7 nm (J mol^{-1});

k'_{HO} is the experimentally obtained fluence based pseudo-first order indirect photolysis rate constant for the probe (cm^2 mJ^{-1});

and the following constants are used: ϵ_{H2O2} = 19 L mol^{-1} cm^{-1}, Φ_{HO} = 1, $k_{HO,C}$ = 5.0 × 10^9 L mol^{-1} s^{-1} for pCBA (Buxton et al. 1988), and $U_{253.7}$ = 471 528 J mol^{-1} for radiation at 253.7 nm. Equation 2 implicitly contains two mutually cancelling unit conversion factors: 1 L/1000 cm^3 × 1000 mW/1 W.

The value of k'_{HO} will be obtained from the overall degradation rate k' observed for the probe compound from:

$$\ell n\,(C/C_o) = -k' \cdot F \quad (3)$$

Where F is the fluence delivered to the sample volume (mJ cm^{-2}), obtained from the product of the exposure time t (s) and the average fluence rate E_{avg} (mW cm^{-2}) delivered to the sample. To obtain E_{avg}, incident fluence rate E_0 can be measured with a calibrated radiometer or with an appropriate actinometry technique (e.g. iodide/iodate described in Bolton et al. 2011) and used with the IUVA protocol and appropriate correction factors (described in Bolton et. al 2015) to determine E_{avg}. A plot of $\ell n\,(C/C_o)$ versus F will allow the determination of k' by linear regression. Because degradation of pCBA is measured, the slope of ln(C/C$_0$) vs. F will be negative, thus the degradation constant k' is the negative of the observed slope.

Generally, direct photolysis, other photoinduced reactions with background matrix and hydroxyl radical reaction from photolysis of H_2O_2 contribute to the degradation of the probe compound. Their rates may be expressed as the sum of individual fluence based pseudo-first order rate constants for hydroxyl radical reaction from H_2O_2 photolysis (k'_{HO}) and all other reactions including direct photolysis of the probe compound under UV light (k'_{UV}):

$$k' = k'_{UV} + k'_{HO} \quad (4)$$

where k'_{UV} and k'_{HO} are both in units of cm^2 mJ^{-1}. Note that both k' and k'_{UV} are obtained experimentally in the presence and absence of H_2O_2, respectively. The value of k'_{HO} is obtained from the difference k' - k'_{UV}.

Once ST is determined experimentally using Equation 2, it must be corrected for contribution to scavenging from H_2O_2 and pCBA added during the experiment using Equation 5. The second order rate constant for the reaction of H_2O_2 with HO$^\bullet$ is 2.7 × 10^7 L mol^{-1} s^{-1} and for the reaction of pCBA with HO$^\bullet$ is 5.0 × 10^9 L mol^{-1} s^{-1} (Buxton et al. 1988).

ST corrected = ST from Eq(2) – [H_2O_2] × 2.7 × 10^7 L mol^{-1} s^{-1} – [pCBA] × 5.0 × 10^9 L mol^{-1} s^{-1} (5)

Approximately 5-10% of H_2O_2 will be consumed in the process. The initial concentration of H_2O_2 can be used in calculations when correcting ST for H_2O_2 contribution without considerably underestimating the ST. The probe compound will also decompose into reaction products. These products are likely to have reaction rate constants with HO$^\bullet$ that are similar to the probe compound's rate constants. However, with higher level of treatment mineralization will occur, which would lead to a non-linear value for ln(C/C$_0$) vs. time or fluence. A UV fluence range must be selected for testing that minimizes the potential for probe compound mineralization, and thus it is recommended that the probe decomposition in the experiment does not exceed 90%. The slope of the ln(C/C$_0$) vs. time or fluence should have $R^2 > 0.95$ with linear regression.

The corrected ST can then be compared to the expected value (calculated) based on the contributions from bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and dissolved organic matter. The second order rate constants for HCO_3^- and CO_3^{2-} are 8.5×10^6 and 3.9×10^8 L mol^{-1} s^{-1}, respectively (Buxton et al. 1988). Literature reports values between 2×10^4 and 3×10^4 L mg-C^{-1} s^{-1} for dissolved organic matter (Westerhoff et al. 1999, Goldstone et al. 2002). Note that if nitrite is detectable, its contribution to ST may also be estimated using the rate constant 1.0×10^{10} M^{-1}s^{-1}. A considerable difference between the measured and calculated value may indicate the presence of other major substances contributing to hydroxyl radical scavenging.

As noted, the probe compound will also contribute to ST and this contribution must be minimized such that ST is essentially constant. However, a sufficiently high concentration of the probe should be present initially such that the degradation kinetics may be adequately captured during an irradiation of reasonable duration (e.g. an irradiation time of 30 s with 1 s of error will have 3% potential error in fluence calculation, while an irradiation of 20 s will have 5% potential error, so irradiation time of at least 30 s per fluence increment is recommended) . A dynamic range of at least an order of magnitude between the limit of quantitation (LOQ) and the spiked probe concentration is desirable. Thus, selection of the initial probe concentration ($[P]_i$) should satisfy the two conditions: $k_P[P] \ll$ ST and $[P]_i \gg LOQ$. If, for example, the water chemistry predicts a value of ST of approximately 5×10^4 s^{-1}, and the method of analysis for pCBA has an LOQ of approximately 0.01 µM, then a $[P]_i$ of 1.0 µM will satisfy both conditions and contribute about 10% to the value of ST at the start of irradiation. In samples with low background scavenging term (e.g., groundwater) it may not be achievable for <10% of the scavenging to be contributed by the probe within the analytical limitations. Each laboratory must assess their detection limits for the scavenging term based on the probe and the analytical method used.

While the protocol is based on pCBA as a probe for HO•, the protocol users may use alternative probes provided that the probe meets the following criteria. The alternative probe should have a well-established k_{HO} that could be referenced to at least two independent sources in agreement. It is recommended that it is additionally verified against pCBA using competition kinetics (at a set temperature and pH). A good probe should have limited reactions other than with HO• (e.g. singlet oxygen, etc.), which includes stability in contact with H_2O_2 and low quantum yield of direct photolysis. The probe should have an established analytical technique that allows it to be measured at low enough concentration to be useful in this protocol, preferably by HPLC with UV detection or a simpler technique. The probe should also have adequate aqueous solubility (at least 100 µM at circumneutral pH). Minimizing UV absorbance of the probe at 254 nm is also important. Alternative probes that fit most or all of these criteria include carbamazepine, sucralose, nitrobenzene (volatilization needs to be controlled), methylene blue, benzoic acid (HPLC detection of product) and terephthalic acid (fluorescence detection of product).

3. Apparatus

a. *Filtering apparatus* with reservoir, coarse fritted disk as support for single use 0.45 µm pore diameter filter and suction flask to which a vacuum may be applied and with sufficient volume for sample size selected. Binder-free glass fiber filters should be used for sample filtration to avoid leaching organic carbon from the filter into the sample. Glass-fiber filters should be

prepared by rinsing them with 100 mL of ultrapure water under vacuum pull and heating them in a furnace at 550 F for 1 hour.

b. *UV collimated beam apparatus*, using an enclosed and fan cooled low pressure mercury vapor lamp type apparatus as described in Appendix C of the USEPA Ultraviolet Disinfection Guidance Manual (USEPA 2006) with appropriate correction factors determined (Bolton et al. 2015). A collimation distance to the sample should be at least four times the lamp aperture diameter. Include at least one magnetic stir plate (a second one will be needed if a dark control is run simultaneously rather than sequentially with the irradiated sample) and at least one jack stand or similar to adjust the height of the sample under the collimated beam.

c. UV radiometer, NIST traceable instrument such as International Light Inc. Model 1700/SED 240/W or equivalent. Should be calibrated within 12 months of use.

d. Crystallization dishes, cylindrical borosilicate glass vessels with an outer diameter smaller than the aperture of the collimated beam, thereby allowing irradiation of the entire liquid surface at the intended distance from the lamp. Suggested to be able to hold a volume of at least 50 mL sample, resulting in a liquid depth of no more than 1 to 2 cm to minimize any error that may be introduced by light filtering effect in low transmittance samples and ensure thorough mixing. It is recommended that the product of sample depth and absorbance at 254 nm does not exceed 0.7. The freeboard above the liquid should be minimized to avoid reflection of the light from the side walls of the vessel into the sample.

e. Teflon coated magnetic spin bars, various sizes, at least two, with minimal thickness relative to the depth of the sample irradiated (larger units may be used for solution preparations). The stir bar should be fully covered by water and not create a surface disturbance in irradiated samples. A very large stir bar in a shallow sample can introduce a considerable error.

f. *High-performance liquid chromatograph (HPLC)*: A system capable of reproducibly injecting up to 100 µL of sample and using two solvents in any combination at a constant flow rate in range of approximately 0.2 to 1 mL/min. A column heater is desirable to maintain the column above room temperature during analysis (30 – 35 ^{o}C). Detection method specifics would depend on the probe compound used.

   i. *Analytical column:* Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Standard Methods 6610B.6) may be used. A compatible guard column is recommended.

   ii. *Detector*: UV detector capable of detection within 200 to 300 nm range. Mass spectrometer may be used, depending on the probe compound, with method specifics defined by the probe compound.

g. *Spectrophotometer*: for use at 254 nm and 352 nm with 1 cm quartz cuvette cell and cell holder. Cuvettes with higher pathlength (5 or 10 cm) should be used for measurements where absorbance value approaches analytical limitations of the spectrophotometer.

h. *pH meter*, any commercial meter that uses a glass electrode and can be read to 0.05 pH units. Calibrate according to the manufacturer's instructions, including attention to temperature compensation and electrode care.

i. *TOC analyzer*: Standard Method 5310. The persulfate method may be preferred to the combustion method for low DOC samples.

j. *Volumetric flasks*: Class A, various sizes, used for preparation of standards, stock solutions, and samples. Suggested sizes of 5, 10, 100, 200, 500, 1000 mL.

k. *Amber glass bottles,* various sizes of 500 to 2000 mL.

l. *Analytical balance,* capable of weighing accurately to 0.0001 g.

m. Autopipettes, Various sizes. Suggested sizes of 20-200 µL and 100-1000 µL; 1-5 mL and 1-10 mL.

n. Volumetric pipette, 50 mL

o. Refrigerator, capable of maintaining a temperature of 4^{o}C.

p. Thermometer, traceable to NIST certification.

q. Stopwatch

4. Reagents

a. Reagent Water, Purified water with a resistivity > 10 MΩ-cm (Standard Methods 1080).

b. Phosphate buffer 1 M stock, pH 7: Add 5.3 g of monosodium phosphate monohydrate ($NaHPO_4 \cdot H_2O$) and 16.5 g of disodium phosphate heptahydrate ($Na_2PO_4 \cdot 7H_2O$) to a 100 mL volumetric flask, add reagent water to the mark and swirl to dissolve. The pH of the buffer should be verified with a calibrated pH probe.

c. Hydrogen peroxide stock solution, 98 mM: Make 100-fold dilution of analytical grade H_2O_2 (30%) without stabilizers in reagent water.

d. Hydrogen peroxide analysis reagents: Dissolve 20.0 g of potassium hydrogen phthalate ($C_8H_5KO_4$) in 1 L of reagent water and store in amber bottle labeled as Solution 1. Dissolve 66.0 g potassium iodide (KI), 2.0 g sodium hydroxide (NaOH), and 0.20 g of crushed ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 1L of reagent water and store in amber bottle labeled as Solution 2. The solutions may be kept for up to one month, based on the method described by Klassen et al. 1994. Other methods may be appropriate as long as they can measure H_2O_2 with accuracy to 0.1 mg/L and have the linear range of > 10 mg/L.

e. *Bovine catalase stock solution* 100 mg/L, Dissolve 500 mg of lyophilized powder in 500 mL of 10 mM of phosphate buffer and mix thoroughly. Phosphate buffer is prepared by adding 5 mL of the 1 M phosphate buffer stock (4b) to a 500 mL volumetric flask and diluting it to the mark with reagent water. Refrigerate when not in use.

f. *Para-chlorobenzoic acid (pCBA) working stock solution*: Use reagent grade ≥ 99%. Weigh between 15 to 20 mg of pCBA, record weight, transfer to clean 1 L Erlenmeyer flask half filled with reagent water and containing Teflon coated magnetic stir bar. Stir flask contents on stir plate for approximately 20 minutes with no heat applied. Mount a 0.45 µm pore size filter on filtration apparatus, wash it with about 100 mL of reagent water and discard filtrate. Filter pCBA solution under light vacuum and transfer filtrate to clean amber glass bottle. Actual concentration will be determined by HPLC using calibration curve.

g. *Para-chlorobenzoic acid (pCBA) standards*: Prepare stock standard, primary dilution standard, and calibration standards as described in Standard Methods 6610B.4 using methanol for the stock standard and 50:50 water and acetonitrile mix for the calibration standards. Prepare at least five calibration standard solutions over the concentration range of interest. Suggested range spans approximately 0.01 to 1.0 µM (1.5 to 150 µg/L).

h. *Tertiary butyl alcohol stock solution*: Weigh approximately 1.0 g of tertiary butyl alcohol (tBuOH) of reagent grade (≥ 99%) in a small beaker and transfer to 1 L volumetric flask half filled with reagent water, fill to mark with reagent water and mix by inversion. Transfer to amber glass bottle for storage at 4^{o}C for up to one week. Make a 10^{-2} dilution of stock for analysis by TOC analyzer and record reading.

i. *Method verification solution*: To a 200 mL volumetric flask add 200 µL of the H_2O_2 stock, 2 mL of 1 M phosphate buffer solution, 2 mL of pCBA stock, and 2 mL of tBuOH stock, fill to mark with reagent water and invert to mix several times. This will result in a solution of approximately 3 mg/L of H_2O_2, ≤ 1 µM pCBA, and 10 mg/L of tBuOH in a 10 mM phosphate buffer at pH 7. Scale accordingly for alternate volume.

j. *Acetonitrile*, CH_3CN, HPLC grade or better.

k. *Methanol*, CH_3OH, HPLC grade or better.

l. *Aqueous phase for HPLC analysis*: add 650 µL of 85% phosphoric acid or 1000 µL of formic acid to 1.0 L of reagent water (per probe compound analysis method). Verify that final pH is ≤ 2.

m. *Chemical actinometry solution*: potassium iodide (KI), potassium iodate (KIO_3), and sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) prepared according to the protocol described in the supplementary material provided in Bolton et al. 2011.

5. Preparatory Procedures

a. *Sampling and storage*: Obtain a representative sample of source water of at least 2 L volume in clean glass containers rinsed with the water to be collected. Filter and analyze for DOC, alkalinity, pH, and the 1 cm UV transmittance at 254 nm (UVT). Nitrite should be also measured in samples where its presence is expected or suspected, e.g. wastewater, groundwater. Store in amber glass bottle without headspace until analysis. Analyze for ST without delay, but if delay is longer than 2 h then store at 4°C and report length of storage at time of analysis. Hold time of <24 hours is recommended. Samples that are shipped for analysis should be collected without headspace, chilled to 4°C upon collection and shipped overnight on ice. Temperature on receipt should be recorded, and the sample should be processed immediately to stay within the 24 hour holding time. No chemical preservation should be used to avoid altering the scavenging term.

b. *Preparation of stock solutions*: Assemble the stock solutions for H_2O_2, reagent solutions for H_2O_2 analysis (Solutions 1 and 2), pCBA (or alternate probe), and bovine catalase as described above.

c. *Verification of H_2O_2 stock solution*: Produce a 100-fold dilution of H_2O_2 stock. To a 10 mL volumetric flask, add 2.5 mL each of Solution 1 and Solution 2 and mix. Then add 1.0 mL of diluted stock and fill to mark with reagent water. Mix and wait about 1 minute, then measure sample absorbance at 352 nm after zeroing the instrument with reagent water. Repeat the above with the mixture of Solution 1 and Solution 2 that does not contain diluted stock to measure the absorbance of the reagent blank. Calculate the concentration of the diluted H_2O_2 stock in mg/L by subtracting the absorbance of the reagent blank from the sample absorbance and multiplying by 12.854 (Klassen et al. 1994). Repeat this procedure three times. Retain the diluted H_2O_2 solutions for subsequent catalase verification. Additionally, the high concentration stock can be verified spectrophotometrically using ε_{H2O2} of 40 $M^{-1}cm^{-1}$ at 240 nm.

d. *Verification of bovine catalase stock solution*: Using a 100-fold dilution of H_2O_2 stock, decant 50 mL into a beaker and add 500 µL of catalase stock solution and mix. Measure the remaining H_2O_2 after 1, 10 and 30 min. If H_2O_2 is detected after 10 min, produce a new catalase stock solution and repeat this procedure. If H_2O_2 is still detected after 30 min, increase the dose of catalase stock to 100 µL. If after increasing H_2O_2 dose it is still detected after 10 and 30 min, consider replacing the source of bovine catalase.

e. *Chromatographic conditions*: Select a mobile phase composition, flow rate, and detection wavelength(s) that results in analyte retention times between 2 and 15 minutes, baseline resolution of probe and non-target peaks, and adequate dynamic range. Mobile phase composition will consist of acetonitrile or methanol, and an aqueous phase composed of reagent water with pH adjustment. The pH of the aqueous phase should be about 2 pH units away from the pK_a of any acidic analytes. Suggested conditions are as follows; mobile phase of 70% acetonitrile and 30% aqueous phase composed of water adjusted to pH 2.3 to 2.6 using 0.65 mL of 85% phosphoric acid for every 1.0 L of reagent water. Use a flow rate of 1.0 mL/min. Detection occurs at peak absorbance between 220 and 300 nm, using 239 nm for pCBA. Following elution of the probe an increase to 100% organic solvent for 2 min may be used before returning to 70:30 isocratic mode to reduce retention of organic solutes, carryover of

analyte and ghost peaks in subsequent runs. Use of acetonitrile will result in lower detection limit as compared with methanol.

f. *Chromatographic calibration*: Using the calibration standards and the chosen chromatographic conditions, generate a calibration curve by plotting peak area against analyte concentration for each of the calibration standard solutions, using at least three injections per standard. Determine the relative standard deviation (%RSD) at each analyte concentration.

g. *Probe working stock concentration determination*: Prepare a 100-fold dilution of the probe stock solution in water. Analyze by HPLC using at least three injections and use the average peak area and the calibration curve developed to determine the concentration of the original stock solution. Any probe that is used must be stable in the presence of H_2O_2 within the timeframe of the experiments and analysis (no measurable reaction with H_2O_2 in a 24 hour dark experiment).

h. *Determination of incident irradiance*: Turn on the fans and lamp of the collimated beam and wait at least 20 min for lamp temperature to stabilize. Determine the incident irradiance at the surface of the irradiated solution using the calibrated radiometer or actinometry protocol described by Bolton et al. 2011. Express in units of mW cm^{-2}.

i. *Determination of average fluence rate in mixed sample under irradiation*: Using the protocol of Bolton et al. 2015, the measured UVT and depth of the sample in the dish, determine the correction factors (Petri Factor, Water Factor, Divergence Factor, Reflection Factor). Excel spreadsheets are available either from the authors of the referenced protocol or in the Member Zone of the web site of the International Ultraviolet Association (www.iuva.org). Use the incident irradiance and the correction factors to calculate the volume corrected average fluence rate in the mixed sample under irradiation. Express in units of mW cm^{-2}. Note that the addition of H_2O_2 will increase the absorbance slightly (decrease the UVT). Correction of the average fluence rate may be necessary using UVT measured after H_2O_2 addition, though for most drinking water sources this error will be negligible.

j. *Determination of exposure times for the target fluences*: Calculate the exposure times by dividing the target fluence values by the average fluence rate. Suggested target fluences are 200, 400, 600, 800, 1000 mJ cm^{-2}. These may be adjusted in order to best capture the probe degradation kinetics. Refer to 6b to adjust irradiation time if needed.

6. Testing Procedure

a. *Preparation of test solution*: Using a 200 mL volumetric flask and filtered sample at room temperature, fill the flask half full, add 2.0 mL of the pCBA stock solution (100-fold dilution), add 200 µL of the H_2O_2 stock solution (1000-fold dilution), fill to the mark with sample, cap and invert at least three times to mix. Into two crystallization dishes, decant a small portion of the test solution, rinse and discard, then using a 50-mL volumetric pipette transfer 50 mL aliquots of the test solution to the two dishes, and add rinsed stir bars using forceps. Measure and record the depth of the liquid. The target H_2O_2 concentration should be approximately 5-10 mg/L. For

samples with expected high ST that concentration may be increased as needed, but typically not more than to 25 mg/L. It is important to make sure that H_2O_2 contributes a small fraction of the overall ST, so that the decay in H_2O_2 does not affect the linearity of the results. Generally, this can be achieved by assuring that the ratio of H_2O_2 to DOC (on mg/L basis) does not exceed 10.

Preliminary range finding: An initial irradiation run is conducted to determine the range of exposure times for subsequent irradiations required to achieve adequate degradation of the probe for calculation purposes. Using the two dishes containing 50 mL, place one dish on a stir plate under the collimated beam with the shutter closed and adjust height if needed to position the liquid surface to the plane where the incident fluence rate has been determined. Place the other dish on a stir plate shielded from the light to serve as a dark control. Adjust mixing to the highest speed that does not produce significant deformation of the liquid surface. Open the shutter and start stopwatch. Sample 0.5 mL from both dishes at five intervals over a period corresponding with the time to deliver 2000 mJ cm^{-2}, placing samples in labeled HPLC vials containing appropriate amount of bovine catalase (based on calculations performed in 5d). During irradiation, sample from flask for the initial concentration of probe and analyze initial UVT and H_2O_2. At the end of the exposure period, analyze for the final UVT and H_2O_2 remaining in both the irradiated and dark control dishes. From the chromatograms, obtain the probe peak areas A_t at various times, to plot the natural logarithm $\ell n(A_t/A_o)$ vs. exposure time for both irradiated sample and dark control, with A_o the peak area of the unirradiated solution. Use this information to select discrete exposure times for subsequent irradiations such that the probe concentration is reduced by approximately a factor of 2 (50% decrease in peak area) and no more than a factor of 10 (90% decrease in peak area). The probe decay should be measurable at all five exposure times yet not so high where it would considerably alter the chemistry of the solution. If degradation is inadequate, consider increasing H_2O_2 dose or exposure time, and vice versa if too much probe degradation is observed in this experiment. A significant (>10% over the course of the experiment) increase in UVT (bleaching) or decrease in H_2O_2 may also require correction in subsequent irradiations (recalculating delivered fluence based on the changing UVT and H_2O_2 for each exposure). (Note: If repeated sampling is taken from the same dish, assure that the overall sample depth does not change by more than 10% of the initial value.)

b. *Determination of the error range σ for the overall observed rate constant* $(k' \pm \sigma)$: Perform a series of discrete irradiations, including dark controls, using the exposure times obtained from step 5.b., each time preparing the solution as in step 5.a. Repeat each exposure time in triplicate. From each test solution, after transferring aliquots to the two dishes, analyze the unused test solution remaining in the flask to obtain the initial UVT and H_2O_2. Also use the remaining test solution to determine the initial probe peak area (A_o) by withdrawing 0.5 mL and placing it into an HPLC vial containing approximately 5 µL of catalase stock solution Following each exposure time t, close the shutter to stop the irradiation. Withdraw 0.5 mL of sample and place it into an HPLC vial containing 5 µL of catalase stock solution to analyze for the remaining probe concentration to obtain the peak area (A_t). Use the remaining sample in both the irradiated sample and dark control to analyze for final UVT and H_2O_2 in each solution. From the data of $\ell n(A_t/A_o)$ vs. t, determine the slope for the irradiated sample and dark control and their associated error. Subtract the slope of the dark control from that of the irradiated sample

to obtain the overall observed photolysis rate constant (k') in time-based units (s^{-1}). Calculate the uncertainty of the overall rate constant (σ) by adding the standard errors of the two slopes in quadrature.

$$\sigma = \left(\sigma_{overall}^2 + \sigma_{DC}^2\right)^{1/2} \quad (5)$$

Convert k' and σ to fluence based units by dividing by the volume corrected fluence rate found in *5.i*.

c. *Determination of the error range σ_{LC} for the UV exposure rate constant $k'_{UV} \pm \sigma_{UV}$:* Perform a series of discrete irradiations, including dark controls, using the exposure times obtained from step 6.b., each time preparing the solution as in step 6.a but without H_2O_2 addition. Repeat each exposure time in triplicate. From each test solution, after transferring aliquots to the two dishes withdraw 0.5 mL from the unused solution to determine the initial probe concentration (A_o). Following each exposure time t, close the shutter to stop the irradiation and analyze the solution for the remaining probe concentration to obtain A_t for both irradiated solutions and dark controls. From the data of $\ell n(A_t/A_o)$ vs. t, determine the slope for the irradiated and dark control and their associated standard errors σ_{LC} and σ_{DC}, repsectively. Subtract the slope of the dark control from that of the irradiated sample to obtain the UV-induced reactions rate constant (k_{UV}) in time-based units (s^{-1}). Calculate the uncertainty of the UV-induced reactions rate constant (σ_{UV}) by adding the standard errors of the two slopes in quadrature.

$$\sigma_{UV} = (\sigma_{LC}^2 + \sigma_{DC}^2)^{1/2} \quad (6)$$

Convert k'_{UV} and σ_{UV} to fluence based units by dividing by the volume corrected fluence rate found in *5.i*.

d. *Calculate the indirect photolysis rate constant $k'_{HO} \pm \sigma_{HO}$*: Obtain the indirect photolysis rate constant in fluence-based units using $k'_{HO} = k' - k_{UV}$ and the associated standard error σ_{HO} by adding σ and σ_d in quadrature:

$$\sigma_{HO} = (\sigma^2 + \sigma_{UV}^2)^{1/2} \quad (7)$$

e. Compute ST using Equation 2 with the measured values of $[H_2O_2]$ and k'_{HO} , as well the other known values. Estimate the uncertainty in ST using the following:

$$\sigma_{\mathrm{ST}} = \mathrm{ST} \cdot \left\{ \left(\frac{\sigma_{HO}}{k'_{HO}} \right)^2 + \left(\frac{\sigma_{H2O2}}{[H_2O_2]} \right)^2 \right\}^{1/2} \quad (8)$$

f. Correct the value of ST to remove the contribution of H_2O_2 and the probe compound. Report ST as corrected ST ± σ_{ST}

7. Quality Control Checks

a. Using the solutions prepared described in 4.g and 4.h, subject a portion of the *Method Verification Solution* to the same analysis as the unknown for ST determination. Compare the experimentally determined value of ST with that calculated from the known concentration of tBuOH and its $HO^{\bullet}$ rate constant of 6.0×10^8 L mol^{-1} s^{-1} (Buxton et al. 1988). Report the discrepancy as a percentage.

b. Check radiometer calibration using actinometry protocol provided by Bolton et al. 2011.

8. Method Limitations and Interferences

a. This method should not be used with samples containing oxidants such as free chlorine, chloramine or ozone or in samples in which these oxidants were quenched by either organic or inorganic substances such as thiosulfate or bisulfite.

b. Elevated levels of iron may create a photo-Fenton reaction. If the levels of iron are of concern, the user is advised to use additional controls to assess the contribution of the photo-Fenton's process to the overall decay of the probe. This could be achieved with a sample spike that doubles the concentration of the expected interference or any other appropriate method. If probe decay in a spiked vs non-spiked sample exceeds 5%, further corrections would need to be applied to the method. Similar interferences may be observed for high copper concentration.

c. This method is not appropriate for reverse osmosis permeate and similar samples with very low $HO^{\bullet}$ scavenging. Method Limit of Quantification (LoQ) for ST can vary by laboratory based on the analytical method used for quantification of the probe compound. LoQ for background ST would be < scavenging associated with H_2O_2 and the probe compound combined.

d. Some applications of AOP may require very high UV fluences, e.g. 1,4-dioxane decomposition may need fluences as high as 4000 mJ/cm^2. Such high fluences may alter the water matrix and considerably change the ST as a result, for example through oxidation and mineralization of background organic matter or through nitrite build-up in samples containing nitrate. It is important to understand that this protocol measures an initial ST for the sample, but that value can change in the actual treatment at UV fluences higher than those applied in this protocol.

e. When determining exposure times, care should be taken to account for probe volatilization, sample evaporation or heating when exposure time per fluence increment exceeds a few minutes. In such circumstances additional interventions or controls will be necessary.

9. References

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