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Singlet Oxygen Generation for Solution Kinetics: Clean and Simple

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Abstract: The rates of singlet oxygen reactions can be accurately measured in solution without the complications associated with conventional methods of singlet oxygen generation using the technique described in this paper. A suitable sensitizer is immobilized on one surface of a glass plate. The sensitizer-coated plate is placed sensitizer-side down on top of a shallow dish partially filled with a solution of the substrates, leaving a small air space (1–2 mm) between the sensitizer and the solution. Illumination of the plate from above causes singlet oxygen to be formed at the sensitizer-coated surface of the plate. A significant portion of the singlet oxygen formed can diffuse the short distance from the sensitizer to the solution to react with the substrate at the surface of the solution. Since the sensitizer and the substrates are physically separated over a distance too large to allow electron or hydrogen atom transfer, this arrangement eliminates the possibility of most photosensitized reactions that do not involve singlet oxygen and provides a simple and inexpensive source of mechanistically clean singlet oxygen for kinetic studies.

The lowest energy excited singlet state of molecular oxygen is an important intermediate in a large number of chemical reactions of biological, medical, commercial, and environmental significance. Singlet oxygen has been found to mediate photooxidations sensitized by many kinds of molecules under a wide variety of conditions in solids, liquids, gases, and solutions. It has been implicated in the photochemical transformations of air pollutants[1-16] and in the degradation of synthetic polymers.[17-19] Singlet oxygen has been proposed as a useful reagent for specific oxidation of chemical compounds, surface treatment of plastics and rubber, and bleaching paper and textile fibers.[17-19] In humans singlet oxygen may be generated by phagocytic cells as a defense mechanism against microbial invasion, and it may be formed as a side product of some enzymatic reactions.[20-23] In medicine it is produced as a reactive intermediate in the action of the photochemotherapeutic antitumor compound, hematoporphyrin derivative,[24-26] and in the phototherapeutic action of furocoumarins.[27-32] Because of its ubiquitous nature and because

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of its inherently interesting chemical characteristics, singlet oxygen has been extensively studied for several years.[33] Progress in these investigations has been limited at times by the need for a reliable source of singlet oxygen which lacks troublesome side reactions or difficult or complicated operating conditions.

Although a number of methods have been developed for generating singlet oxygen, none are ideally suited for kinetic analysis of singlet oxygen reactions in aqueous solution. For instance, use of dye-sensitized photochemical generation of singlet oxygen for rate measurements requires careful and relatively elaborate data analysis because dyes may also sensitize substrate oxidations that are not singlet oxygen mediated.[22,34-37]

Microwave or radio frequency discharge generation of singlet oxygen is not suitable for kinetic studies because the high flow rate and low pressure of the gas impinging on the solution causes rapid evaporation. This results in cooling of the solution, which is difficult to control, and increases in concentration of the substrates, effects that complicate rate measurements.

Some chemical sources of singlet oxygen, such as potassium perchromate, generate other oxidative species besides singlet oxygen[38] or, like triphenyl phosphite ozonide[39] or hydrogen peroxide/hypochlorite, may react directly with the substrate under some conditions making interpretation of results uncertain. Such reagents are often potent oxidants themselves. Some other chemical sources of singlet oxygen that are mechanistically sound, such as diphenylanthracene endoperoxide, react with hydroxylic solvents or are insoluble in water, making their use in aqueous solution difficult if not impossible.[40] This problem has recently been solved by Saito and Matsuura[41] by using the 1,4-endoperoxide of 3-(4-methyl-1-naphthyl)propionic acid. However, even this reagent is not ideal. The use of any chemical source of singlet oxygen is subject to complications arising from the presence in the reaction of additional reagents and products whose effects are unknown.

It is possible to cause ground-state molecular oxygen to undergo direct electronic transition to the excited singlet state using intense laser light of the appropriate wavelength.[42,43] However, because of the forbidden nature of this transition, very high oxygen pressures are required for reasonable quantities of singlet oxygen to be produced. Consequently, this technique requires relatively expensive apparatus and elaborate safety precautions.

In order to avoid these complications, we have devised a simple, inexpensive method for generating singlet oxygen using photosensitization. This technique avoids the mechanistic ambiguity associated with conventional methods of photosensitized generation of singlet oxygen by physically separating the sensitizer and substrates so that reaction by S-s[44] (non singlet oxygen) mechanisms is impossible.

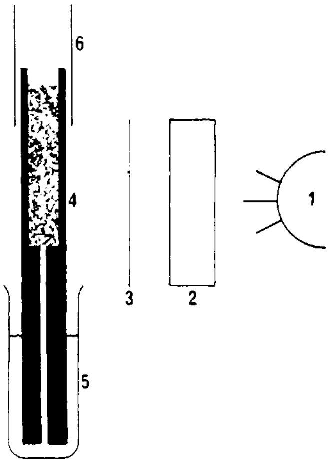

Figure 1. First system tested for gas-phase generation of singlet oxygen: 1, light source; 2, water-filled tank serving as IR/UV filter; 3, high-pass or band-pass filter; 4, glass tube filled with immobilized sensitizer attached to capillary tube; 5, test tube holding the substrate solution; 6, flexible tubing connected to a source of air or gas.

Theory

Photosensitized oxidation can occur by either of two fundamental photochemical mechanisms.[22,34-37] In the S-s[44] or type I mechanism, excited-state sensitizer activates the substrate directly, usually by electron or hydrogen atom transfer. The activated substrate can then react with ground-state oxygen to give photooxidation products. In the S-o or type II mechanism, excited-state sensitizer can generate excited singlet state oxygen by energy transfer. Then singlet oxygen can oxidize the ground-state substrate.

Many dyes which have readily populated, long-lived triplet states can sensitize production of singlet oxygen. Some of the dyes which are most commonly used for photochemical generation of singlet oxygen are Rose Bengal and Methylene Blue. However, it is also possible for many dyes to sensitize other types of photooxidation. Most of the non singlet oxygen photooxidation mechanisms require direct interaction between the sensitizer and the substrate generally by electron or hydrogen atom transfer (S-s) mechanisms. Therefore reaction by non singlet oxygen mechanisms can be minimized by physically separating the sensitizer and the substrate by a sufficient distance to prevent electron or hydrogen atom transfer. Under these conditions the S-o mechanism may still be possible if singlet oxygen can diffuse between the sensitizer and the substrate.

Singlet oxygen can only diffuse 100–200 nm in aqueous solution.[45,46] Therefore if the source of singlet oxygen is in solution, the substrate must be located closer than 200 nm in order to be able to trap a reasonable fraction of the singlet oxygen before it decays. In photosensitized oxidations in solution it is difficult to achieve this while still maintaining sufficient separation of sensitizer and substrate to prevent S-s type reactions. One approach would be to immobilize the substrate on the same support as the

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(44) S-s is used here to replace the mechanism label, "type I", and S-o is used to replace "type II" because it is believed that the S-o and S-s mechanism labels are clearer and more descriptive than the numerical type labels. S-o is used to indicate a reaction path in which products are formed as a result of interaction between excited sensitizer, S, and oxygen, o (e.g., singlet oxygen mediated reactions). S-s indicates a reaction path in which products are formed as a result of interaction between excited sensitizer, S, and substrate, s. (e.g., excited sensitizer activates substrate by hydrogen or electron transfer and activated substrate goes on to react with ground state oxygen). A mechanism could be imagined in which products are formed by interaction of excited sensitizer with both oxygen and substrate. Such a mechanism could be labelled S-o-s or S-s-o depending on the sequence of reaction steps.

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sensitizer, but then simple solution kinetics could not be measured and it would be difficult to design immobilization procedures that would yield the optimum separation of substrate and sensitizer.

Therefore we chose to design a different method in which singlet oxygen is generated in the gas phase above the solution. Singlet oxygen has a much longer (100–10000×) lifetime in the gas phase than it does in solution, and therefore the distance between the source of singlet oxygen and the substrate can be greater in the gas phase. In the first system that we tested, silica gel particles were coated with sensitizer and placed in a glass tube with a capillary attached at one end as shown schematically in Figure 1. The capillary was immersed in the substrate solution. The object was to generate singlet oxygen by illumination of the immobilized dye and to sweep the singlet oxygen into solution with a stream of air or inert gas flowing at 1–10 mL/min. The capillary was used to minimize the flow rate needed to transfer the short-lived singlet oxygen to the substrate before the singlet oxygen decayed and to separate the singlet oxygen generation zone from the substrate solution so that the sensitizer could be illuminated without illuminating the substrate solution. We found however that the solid surfaces of the silica gel and capillary were so efficient at quenching singlet oxygen that at reasonable gas flow rates, essentially none of the singlet oxygen was able to escape the generation zone.

This was not the first time gas-phase singlet oxygen had been studied. Generation of gas-phase singlet oxygen has been demonstrated many times in the past, but until recently all but one investigation involved generation of singlet oxygen at reduced pressure.[47,48]

Recently a method for singlet oxygen generation at atmospheric pressure has been designed and used by Eisenberg, Snelson, Butler, Vetman, and Murray[47,48] to study reactions relevant to atmospheric transformations of pollutants. In this method the inside of a glass tube is coated with Rose Bengal. Oxygen or air is passed through the tube at high flow rates (50–60 L/min) while the tube is illuminated with intense visible light. A light baffle, reaction zone, and product traps or optical detector are connected to the outlet of this tube. Singlet oxygen generation has been demonstrated in this system by detection of the weak but characteristic emission of singlet oxygen in the near-IR and by its characteristic reactions with known singlet oxygen acceptors and quenchers. The high flow rate of the gas is necessary in this system to transport the short-lived singlet oxygen to the reaction vessel before it decays. While this system is very useful for studies of gas-phase reactions, it is not a convenient source of singlet oxygen for solution reactions. The high flow rate of the gas impinging on the solution will cause sample cooling and solvent evaporation similar to that experienced in the radio frequency discharge generation of singlet oxygen mentioned above.

Since we were interested in solution kinetics rather than gas-phase reactions, we needed a different approach. Our calculations suggested that singlet oxygen should be able to diffuse a short distance in air at atmospheric pressure. Therefore we designed a system that placed the immobilized sensitizer within this limiting distance of the reaction solution.

Calculations based on the Einstein–Smoluchowski equation for net displacement due to random molecular motion[49] suggest that as much as half of the singlet oxygen generated in air at one atmosphere pressure may be able to diffuse more than a millimeter before it is quenched. Placing a sensitizer 1 mm in the air above the reaction solution should therefore allow a significant portion of the singlet oxygen generated to reach the surface of the solution and react. The proper location of the sensitizer above the substrate solution can be achieved by attaching sensitizer-coated silica gel particles to one side of a glass plate. The glass plate is then placed dye-side down on a shallow (1–4 mm deep) dish. The dish is partially filled with the substrate solution so that an air space less than 1.5 mm thick exists between the plate and the solution as schematically illustrated in Figure 2. When the plate is illuminated from above, singlet oxygen is generated at the surface of the plate. The singlet oxygen can then diffuse to the surface of the solution and react with the substrate.

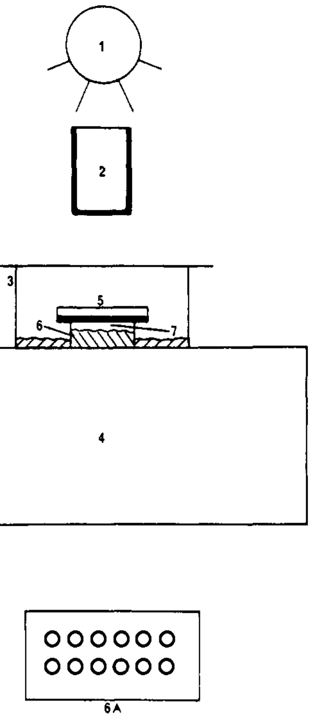

Figure 2. System for singlet oxygen generation for solution kinetics: right diagonal (///), buffer or solvent; left diagonal (\\\\\\), substrate solution; 1, light source, 150-W tungsten narrow spot light; 2, water-filled beaker serving as IR/UV filter; 3, large petri dish and cover; 4, cooling plate; 5, sensitizer-coated plate, sensitizer-side down; 6, small shallow dish less than 4 mm deep; 6A, Boerner slide; 7, air space between sensitizer and substrate solution, less than 1 mm thick.

Some sensitizers can generate other reactive species that under certain conditions might mimic singlet oxygen. One way this system can be tested to determine the identity of the species causing substrate oxidation is by measuring the dependence of the rate of substrate oxidation on the distance separating the substrate solution and the sensitizer.

Because the lifetime of singlet oxygen in water is very short (2–4 μs), the singlet oxygen oxidation of substrate in this system will take place only in a very thin section of the surface of the solution. Because the rate of generation of singlet oxygen in the experiments described here is slow, diffusion of substrate into the reaction zone will be much faster than the rate of oxidation of substrate. This means the concentration of substrate in the reaction zone will tend to be the same as the concentration of substrate in the bulk solution. Therefore, the rate of change of concentration of substrate in the reaction solution will depend on the amount of solution. This can be taken into account by defining a rate constant that relates dA, the *change* in the *amount* of A reacting, with $[A]$, the *concentration* of A in the reaction solution, and $[^1O_2]_{s-s}$, the steady-state concentration of singlet oxygen at the surface of the solution, as shown in eq 1, where $[A] = A/V$,

$$-\mathrm{d}A/\mathrm{d}t = k_R[A][^1O_2]_{s-s} \qquad (1)$$

V = the volume of the substrate solution, and k_R = an unconventional rate constant for reaction of A with $^1O_2$ (k_R has units of L^2 mol^{-1} s^{-1}). The unconventional rate constant is necessary because the distance separating the sensitizer and substrate solution was varied experimentally by varying the volume of solution in the dish and because the rate of change of the concentration of

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[47] Eisenberg, W. C.; Snelson, A.; Butler, R.; Veltman, J.; Murray, R. W. *Tetrahedron Lett.* **1981**, 377–380 and references therein. This and the following reference include an excellent discussion of the early work of gas-phase generation of singlet oxygen.

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[49] Kennard, E. H. "Kinetic Theory of Gases"; McGraw-Hill: New York, 1938; pp 286–287.

the substrate depends on the volume of the reaction solution. Because reaction is only occurring at the (two-dimensional) surface of the solution, the amount of substrate reacting in a given time will be volume independent, while the concentration change is volume dependent.

The concentration of singlet oxygen at the surface of the solution will be constant as long as light intensity remains constant, and the sensitizer is not degraded. Therefore, multiplying both sides of eq 1 by $1/V$, rearranging, and integrating gives eq 2. A

$$V \ln ([A]_{t=0}/[A]_t) = [{}^1O_2]_{s-s} k_R t \tag{2}$$

pseudo-first-order rate constant for oxidation of substrate, A, by singlet oxygen is defined as k'_r such that

$$\ln ([A]_{t=0}/[A]_t) = k'_r t \tag{3}$$

k'_r can be evaluated from a plot of ln $[A]$ vs. time. If k'_r is evaluated for two different volumes of substrate solution (two different distances, x_1 and x_2, separating the sensitizer and the substrate solution) under otherwise identical conditions, the ratio of the two rate constants determined will be given by eq 4 or, in

$$[Vk'_r]_{x_1}/[Vk'_r]_{x_2} = ([{}^1O_2])_{x_1}/([{}^1O_2])_{x_2} \tag{4}$$

other words, the rate of substrate oxidation will be directly proportional to the concentration of singlet oxygen at the surface of the solution and inversely proportional to the volume of the solution.

The singlet oxygen concentration in air a distance, x, from its source, $[{}^1O_2]_x$, can be calculated[50] from eq 5, where k'_q = the

$$[{}^1O_2]_x/[{}^1O_2]_{x=0} = \exp(-k'_q x^2/2D) \tag{5}$$

pseudo-first-order rate constant for singlet oxygen quenching in solvent saturated air and D = the diffusion constant for molecular oxygen in air, assuming pseudo-first-order decay. The pseudo-first-order rate constant, k'_q, for decay of singlet oxygen in air at 1 atm can be calculated from the published second-order rate constants for quenching of singlet oxygen by molecular oxygen, nitrogen, and water in the gas phase.[51] Substituting eq 5 into eq 4 gives eq 6 which describes the dependence of the rate constant for oxidation of substrate, k'_r, on the distance between the immobilized sensitizer and the surface of the solution, x.

$$[V(k'_r)]_x/[V(k'_r)]_{x=0} = \exp(-k'_q x^2/2D) \tag{6}$$

The distance dependence of k'_r will be characteristic of the lifetime of the reactive species responsible for substrate oxidation. If the observed oxidation of the substrate, A, in this system is due to dye contaminants in solution, arising, for instance, from particles falling from the sensitizer-coated plate, then the rate of A oxidation should not depend on the distance between the sensitizer and the solution. If, on the other hand, the oxidation of A is due to reaction of singlet oxygen generated at the surface of the plate and diffusing to solution, then a plot of the relative rate constant for substrate oxidation, $[Vk'_r]_{x=0}/[Vk'_r]_x$, vs. distance between the sensitizer and solution, x, should fit eq 6. And a plot of ln $[V(k'_r)_x]$ vs. x^2 should give a straight line. The half-life of the reactive species in the atmosphere above the solution can be calculated from the slope of this line using eq 7. This value can be compared to the

$$t_{1/2} = 0.693/(2D\,\text{slope}) \tag{7}$$

value calculated from the published rate constants for quenching of singlet oxygen by the components of air (see Results).[51]

Materials and Methods

2,5-Bis(hydroxymethyl)furan was obtained from Aldrich Chemical Co. and purified by preparative HPLC on a Whatman Magnum 9-C_{18} reverse-phase column, eluting with water/methanol, 90:10 (v/v). Deoxyguanosine, L-histidine, L-tryptophan, Rose Bengal, and Methylene Blue were purchased from commercial sources. Purity of substrates was monitored by using reverse-phase HPLC on Whatman ODS-3 columns, eluting with water/methanol, 93:7 (v/v).

Immobilized sensitizers were purchased in the form of Sensitox I and II[52-54] from Hydron Laboratories, Inc., New Brunswick, N.J., or prepared by coating silica gel, glass beads, or alumina with the appropriate sensitizer. The support was coated with the sensitizer either by covalent attachment or physical adsorption. Covalent attachment was achieved by using the method of Neckers et al. for Rose Bengal[55] or the method described by Lewis and Scouten for Methylene Blue.[56] Diazotized glass beads purchased from Pierce Chemical Co., Rockford, Il., can be substituted for the diazotized glass beads prepared according to Lewis and Scouten. It is necessary to wash the glass beads exhaustively with methanol after reaction with Methylene Blue in order to remove all traces of unbound dye. The beads with Methylene Blue attached are golden brown in color after thorough washing. Physical adsorption was accomplished as described by Nilsson and Kearns,[57] except that smaller amounts of sensitizer were used. A weighed amount of sensitizer was dissolved in 3 mL of absolute methanol, 1 g of the support added, and the solvent evaporated on a rotary evaporator at 40 °C.

Glass plates were coated with immobilized sensitizer by first coating one side of the plate with a thin layer of "Sanford's" or "Carter's" rubber cement "for sticking paper" and allowing it to dry for approximately 1 h in a dust-free area. The immobilized sensitizer was then sprinkled on the plate until the plate was thoroughly covered with particles. Loose particles were poured off, and the particles which were not firmly bound to the plate were removed with a fast stream of compressed air. Sensitizer was also immobilized by placing a solution of the sensitizer in methanol on the surface of a glass plate which had been roughened by grinding with 400 mesh carborundum. Evaporation of the solvent gave sensitizer immobilized directly on the surface of the plate. However, it is difficult to obtain a uniform coating with the last procedure. A somewhat better coating was obtained by spraying a fine mist of a 1-mg/mL solution of Rose Bengal in acetone onto the ground surface of a glass plate.

The top halves (lids) of small plastic petri dishes (40 by 4 mm) purchased from Falcon Plastic Co. or "Boerner" microscope slides purchased from Arthur H. Thomas Co. were used to hold the sample solutions. The "Boerner" slide had 12 wells, each of which had a total volume of approximately 200 μL. The petri dish lid had a maximum volume of 5.6 mL. Use of a shallow dish to hold the solution favors faster reaction rates because the ratio of surface area to volume of solution is high.

Singlet oxygen oxidations were performed by placing 5 mL of the solution of the substrate in the petri dish, or 160–175 μL of the solution into a well of the Boerner slide. The sensitizer-coated plate was then placed dye-side down on top of the dish or plate as illustrated in Figure 2. This leaves a distance of 0.48 mm between the solution and the sensitizer in the petri dish (inside radius = 2.0 cm, 5.6 mL maximum volume) and a smaller distance in the case of the Boerner slide. The sensitizer is illuminated from above with the beam of a 150-W spotlight. A water-filled beaker is used as a filter to eliminate infrared radiation. The distance between the sensitizer and solution was varied by varying the volume of solution in the petri dish. The greatest separation was achieved by placing 2 mL of the substrate solution in a deeper petri dish which was approximately 10 mm deep.

The dish or slide containing the solution of the substrates was placed in the bottom of a large glass petri dish (140 by 20 mm). The rim of this

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(50) The average distance, x, a molecule diffuses in time, t, is given by the Einstein–Smoluchowski equation (see ref 49): $x^2 = 2Dt$, when D = the diffusion constant. Decay of singlet oxygen in air will be a pseudo-first-order process such that $[{}^1O_2]_t/[{}^1O_2]_{t=0} = \exp(-k'_q t)$, where k'_q is the pseudo-first-order rate constant for quenching of singlet oxygen by the component of air. Combining these two equations gives the distance dependence of the singlet oxygen concentration, eq 5, as given in the text.

(51) $t_{1/2} = 0.693/k'_q$, where $k'_q = k_q^{O_2}[O_2] + k_q^{N_2}/[N_2] + k_q^{H_2O}[H_2O]$. k_q^X is the pseudo-first-order rate constant for quenching of singlet oxygen by X. k_q = 1400 M^{-1} s^{-1} for molecular oxygen, 40 M^{-1} s^{-1} for molecular nitrogen, and 8400 M^{-1} s^{-1} for water vapor in water saturated air at 5 °C. (The vapor pressure of water at 5 °C is 6.5 mmHg.) Most organic solvents have a higher vapor pressure than water but most organic solvents are also poorer quenchers of singlet oxygen than water. Therefore, substitution of an organic solvent for water in the substrate solution will give a higher concentration of quencher in the atmosphere between the sensitizer and the substrate solution, but the rate of singlet oxygen quenching may actually be lower and the diffusion of singlet oxygen to the substrate solution should still be possible in most cases. See for example the results obtained with methanol/water (50:50 v/v) as solvent in Table III.

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Table I. Values of Volume-Corrected Rate Constants for Oxidation of the Substrate 2,5-Bis(hydroxymethyl)furan Varying the Distance between the Sensitizer and the Substrate Solution[a]

		Vk'_r		
V, mL	x, cm	A	B	C[b]
	0	0.764[c]	0.746[c]	0.235[c]
5	0.0478	0.736	0.659	0.234
4	0.127	0.516	0.312	
3	0.207	0.179		0.083
2	0.286	0.076		0.041
2[d]	0.694	0.002[c]	0.002[c]	0.002[c]
		Singlet Oxygen Half-Life Calculated from Above Data[f]		
		0.064	0.035	0.086

[a] Abbreviations: k'_r = apparent pseudo-first-order rate constant for oxidation of the substrate; V = volume of reaction solution; x = distance separating sensitizer and solution, A = 2,5-bis(hydroxymethyl)furan. Conditions: A, $[A] = 10^{-4}$, sensitizer = Rose Bengal covalently bound to glass beads, 10-h illumination; B, $[A] = 10^{-3}$ M, sensitizer = same as in no. 1, 10-h illumination; C, $[A] = 10^{-4}$ M, sensitizer = Methylene Blue physically adsorbed to glass beads at 2.5 mg of dye/g of beads, beads are 37-47 μm in size with 100-Å pore size, 6-h illumination. [b] Higher uncertainty due to small relative changes in $[A]$. [c] $0 < Vk'_r < 0.02$, this value not included in half-life calculations because the change in $[A]$ was too small to give an accurate value. [d] 10-mm deep dish used instead of 4 mm. [e] Taken as the y intercept of plots of ln (Vk'_r) vs. x^2. [f] Calculated from the slope of plots of ln (Vk'_r) vs. x^2 (see text).

petri dish was ground flat by using carborundum so that a glass plate placed on top of the dish would seal it. A few milliliters of the solvent for the reaction was placed in this dish to saturate the atmosphere so that evaporation would be minimized. This assembly was placed on a Thermoelectrics Unlimited, Inc., Model SK 12 cooling plate and kept at 0-10 °C during the reaction to prevent condensation on the sensitizer-coated glass plate and to minimize the vapor pressure of water above the solution. Water is a better quencher of singlet oxygen than either nitrogen or oxygen.[58,59]

The progress of the reaction was monitored by using reverse-phase HPLC (high-performance liquid chromatography) on a C_{18} column. Elution was performed with a Waters Model M6000 pump using water/methanol, 93:7 (v/v), except when histidine was being analyzed, in which case, 0.005 M sodium octanesulfonate, purchased from Eastman Kodak, was added. Substrates were detected by using a Schoeffel Model SF 770 variable-wavelength detector set at appropriate wavelengths. The areas or heights of peaks corresponding to the reactants were substituted for concentrations in eq 6.

The formation of superoxide was monitored by measuring the rate of formation of nitro blue diformazan using a 2×10^{-3} M solution of nitro blue tetrazolium in pH 8 phosphate buffer. Nitro blue diformazan was formed for comparison by reducing nitro blue tetrazolium with superoxide generated by illuminating a solution of riboflavin and EDTA.[60,61]

Results and Discussion

Table I gives the results of oxidation of substrate, A, varying the distance separating the sensitizer and the solution. The relationship between the distance separating the sensitizer and the solution and the rate of oxidation of the substrate is plotted in Figure 3. The circles, squares, and triangles represent the experimental data, and the solid curve (no. 2) represents the predicted relationship for a diffusing oxidant with a half-life equal to singlet oxygen in water-saturated air at 5 °C and 1 atm pressure. The y axis is $[V(k'_r)]_x/[V(k'_r)]_{x=0}$. Since it is impossible to obtain a valid point for $x = 0$ because the sensitizer and the solution would be in contact, this value is obtained (by extrapolation) as the exponential of the y intercept of a plot of ln $[V(k'_r)]$ vs. x^2 (see eq 6) for each set of conditions. The x axis is the distance separating the sensitizer and solution, x. Dotted curves nos. 1 and 3 are drawn for comparison and represent the shape of the relationship for diffusing oxidants with half-lives 10 times and 0.1 as great as singlet oxygen, respectively. As seen in this plot the shape of the curve generated by the experimental results agrees reasonably well with the curve expected for singlet oxygen. Because immobilized Methylene Blue was a poorer sensitizer than Rose Bengal, the extent of oxidation of the substrate, A, sensitized by Methylene Blue was smaller. Consequently the relative error for the results obtained with Methylene Blue is higher. This probably accounts for the poorer fit of the Methylene Blue data (circles on the graph) to the theoretical curve.

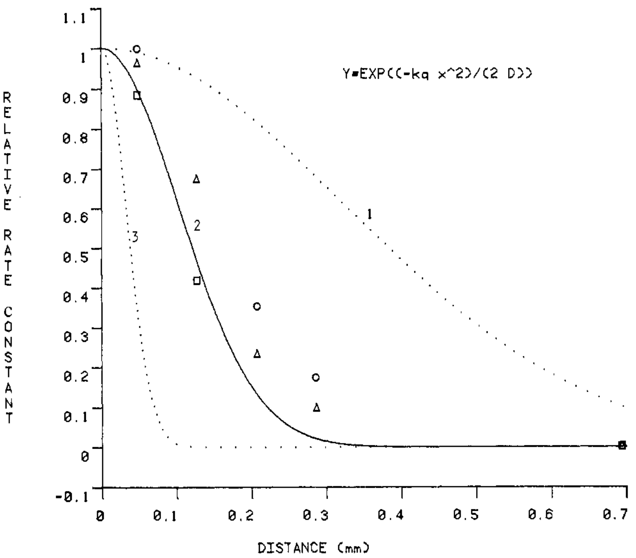

Figure 3. Relative rate constants for oxidation of 2,5-bis(hydroxymethyl)furan vs. the distance separating the sensitizer and the surface of the substrate solution: curve 1, predicted relation for a diffusing species with a half-life 10 times as long as singlet oxygen; curve 2, predicted relation for a diffusing species with a half-life the same as singlet oxygen; curve 3, predicted relation for a diffusing species with a half-life 0.1 as long as singlet oxygen; triangles, rate constants measured by using Rose Bengal coated silica gel as the sensitizer and 10^{-4} M 2,5-bis(hydroxymethyl)furan as substrate; squares, rate constants measured by using Rose Bengal coated silica gel as the sensitizer and 10^{-3} M 2,5-bis(hydroxymethyl)furan as substrate; circles, rate constants measured by using Methylene Blue coated silica gel as the sensitizer and 10^{-4} M 2,5-bis(hydroxymethyl)furan as substrate.

The values determined for the half-life of singlet oxygen from the slope of the linear regression line drawn through plots of ln $[V(k'_r)]$ vs. x^2 using the experimental data agrees reasonably well with the value calculated from published information.[62] Correlation coefficients for such linear plots of the data in columns 1 and 3 in Table I (deleting the point for the greatest distance in each case because the change in $[A]$ was negligible)[63] are 0.983 and 0.991, and respectively. The half-life determined by using immobilized Rose Bengal is 0.064 ± 0.03 s with 10^{-4} M substrate and 0.035 ± 0.01 s with 10^{-3} M substrate. The value determined by using immobilized Methylene Blue is 0.086 ± 0.04 s.[62] The value calculated from published data[51,58,59] on the rate of quenching

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(58) Ogryzlo, E. A. "Singlet Oxygen"; Wasserman, H. H.; Murray, R. W., Eds.; Academic Press: New York, 1979, p 47.

(59) Gollnick, K "Singlet Oxygen: Reactions with Organic Compounds and Polymers"; Ranby, B.; Rabek, J. F., Eds.; John Wiley and Sons: New York, 1978; p 112.

(60) Beauchamp, C.; Fridovich, I. *Anal. Biochem.* **1971**, *44*, 276-278.

(61) Nitro blue diformazan is an intensely purple chromophore formed by reduction of the pale yellow nitro blue tetrazolium.

(62) The accuracy of the determination of the half life of singlet oxygen in air by this method depends on the accuracy of measurement of the distance between the sensitizer-coated plate and the substrate solution. The uncertainty in this measurement is estimated to be ±20%. The 20% uncertainty in the distance imposes 40% uncertainty on the determination of the half life of singlet oxygen because of the square relation between these two parameters.

(63) The data obtained at the greatest distance between the sensitizer and solution was not included in calculating the lifetime of singlet oxygen because the very small change (essentially zero within experimental error) in the concentration of A under these conditions makes the uncertainty associated with this point very high. This point is of value, however, in the plot in Figure 1 since it demonstrates that the "background or control" reaction rate is negligible.

Table II. Comparison of Sensitizer Efficiency[a]

sensitizer	mode of attachment	type of support	pore diameter, Å	particle size, μm	mg of sensitizer/ g of support	ref[b,f] efficiency	ref
Rose Bengal	C	G	100	37-74	ND	0.52	*b*
Rose Bengal	C	G	500	37-74	ND	0.35	*b*
Rose Bengal	C	G	500	125-177	ND	0.12	*b*
Rose Bengal	C	S	60	60-120	ND	0.56	*b*
Rose Bengal	C	S	60	TLC powder	ND	0.13	*b*
Rose Bengal	C	F	190	63-125	ND	0.07	*b, c*
Rose Bengal	A	G	100	37-74	2.5	0.55	
Rose Bengal	A	G	500	125-177	2.5	1.0	
Rose Bengal	A	G	500	125-177	25.0	0.02	
Rose Bengal	A	P-G			3.5 μg/cm²	0.15	
Rose Bengal powder applied directly to the rubber cement						0.10	
Sensitox I	C	P				0.43	*d*
Sensitox II	C	P				0.04	
Methylene Blue	C	G	500	125-177	ND	0.18	*b*
Methylene Blue	A	G	500	37-74	1.0	0.24	
Methylene Blue	A	G	500	37-74	2.5	0.51	
Methylene Blue	A	G	100	37-74	2.5	0.26	
Methylene Blue	A	F	190	63-125	2.5	0.31	
Methylene Blue	A	A-A			2.5	0.46	*e*
Methylene Blue	A	A-N			2.5	0.74	*e*
Methylene Blue	A	A-B			2.5	0.26	*e*
Methylene Blue	A	P-G			3.5 μg/cm²	0.10	

[a] Abbreviations: C = covalent; A = physical adsorption; G = Pierce glass beads; S = silica gel; F = "Fractosil" brand silica; P = polymer; A-A = acidic alumina; A-N = neutral alumina; A-B = basic alumina, P-G = ground glass plate, ND = not determined. [b] Reproducibility of preparations of covalently attached sensitizers was poorer than that of physically adsorbed preparations. [c] This was only prepared once and was much darker in color than the other rose bengal preparations. Its low efficiency may be anomalous. [d] Sensitox was the most resistant to bleaching of the sensitizers tested. [e] Alumina did not adhere to rubber cement as well as did the silica or glass supports. The alumina-based sensitizers bleached faster (within 2 h) than other preparations. [f] Relative efficiency calculated as the ratio of the apparent pseudo-first-order rate constant for substrate oxidation, k'_r, for the given sensitizer to k'_r for the best sensitizer.

of singlet oxygen by oxygen, nitrogen, and water in the gas phase is 0.041 s.

The results indicate that the oxidation of the substrate in these experiments is due to reaction with a species generated at the surface of the sensitizer-coated plate, which has a lifetime identical with or very similar to that of singlet oxygen. This strongly supports the conclusion that substrate oxidation is in fact due to reaction with singlet oxygen generated by the sensitizer-coated plate rather than to reactions sensitized by dye contaminants in solution. Such contaminants, which might have come from particles falling from the plate, from volatile sensitizers arising from photolysis of the immobilized sensitizer and diffusing into solution, or from dye contaminants in the solvents or reagents used to make the solutions, are ruled out by this experiment. This experiment also rules out substrate degradation by any diffusing species with a half-life in air substantially different from that of singlet oxygen.

While photolytic formation of a volatile sensitizer with a half-life similar to that of singlet oxygen can be imagined, these experiments would require that such species formed from photolysis of both Rose Bengal and Methylene Blue have a similar half-life of decay, even though these two sensitizers differ substantially in structure. This seems unlikely.

In order for this system to be generally applicable, it should yield singlet oxygen as the sole reactive product. Although the arrangement described here prevents S-s reactions, another non singlet oxygen reaction is still theoretically possible, i.e., reaction involving intermediacy of superoxide. Superoxide ion (or hydroperoxyl radical) can be photochemically generated by transfer of an electron from excited-state sensitizer to molecular oxygen, but since neither superoxide nor hydroperoxyl radical is expected to have gas-phase lifetimes similar to singlet oxygen, intermediacy of these species in the reaction of the furan substrate is ruled out by the experiment described above. Furthermore, superoxide does not react with deoxyguanosine,[64] while deoxyguanosine is readily oxidized in this system at pH 10. Therefore, the intermediacy of superoxide as a mechanism for the oxidations observed in this system is ruled out. But this does not eliminate the possibility that superoxide or hydroperoxyl are being generated but are not contributing to substrate degradation.

Superoxide production is easily detected by using nitro blue tetrazolium.[63] Pale yellow nitro blue tetrazolium is rapidly reduced to the intensely blue nitro blue diformazan in neutral aqueous solution. When a solution of nitro blue tetrazolium was used in this system, the rate of formation of nitro blue diformazan was found to be too slow to measure—at least 100 times slower than oxidation of singlet oxygen acceptors under the same conditions. It would be necessary to measure the lifetime of superoxide in this system in order to quantitatively evaluate the lower limit of superoxide production, but it must be relatively low; probably less than 1% of the yield of singlet oxygen.

In order to run several reactions at a time, solutions were placed in the wells of a Boerner slide. Since only 20 μL of solution was needed for kinetic analysis by HPLC, the small volume (165 μL) contained in the wells of the slide was not a disadvantage. In fact, because the ratio of surface area to volume was high for this arrangement, reaction rates were relatively fast. Up to 50% oxidation of BHMF was observed within 1 h in this system using the most efficient immobilized sensitizer.

It should be noted that the rate of substrate oxidation in these experiments is very slow. With use of similar amounts of sensitizer dissolved in solution and similar illumination intensities, the substrate would be completely oxidized within minutes, whereas with the system described here only half the substrate is oxidized in 1 h under optimum conditions.

With use of the Boerner slide, the efficiency of several different sensitizer preparations was determined. The results are presented in Table II. These results show that in general immobilized Rose Bengal is a more efficient sensitizer than immobilized Methylene Blue under the conditions used in these experiments. This contrasts with the similar quantum yields of singlet oxygen given by these two sensitizers in solution. The difference may reflect the effects of immobilization on a solid surface.

The results in Table II also show that dyes immobilized on silica or glass beads with larger pore sizes seem to give higher rates of oxidation. This is in spite of the fact that supports with larger pore size have less surface area and therefore less dye available for generating singlet oxygen. The greater sensitizer efficiency is most likely due to the greater ease with which singlet oxygen can escape the larger pores. In general, solids are much better

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(64) Cadet, J.; Teoule, R. *Photochem. Photobiol.* **1978**, *28*, 661-667.

Table III. Relative Rates of Substrate Reaction with Singlet Oxygen

substrate	substrate concn, M	rel rate	solvent	sensitizer
BHMF	10^{-4}	1.0	0.01 M sodium carbonate, pH 10	2.5 mg of MB/g on 10-nm pore, 37-74-μm diameter glass beads
dGuo	10^{-4}	0.4 ± 0.1		
His	10^{-4}	1.0	water/methanol, 50:50 (v/v)	2.5 mg of RB/g on 50-nm pore, 37-74-μm diameter glass beads
Trp	10^{-4}	0.65 ± 0.15 (0.57[65])		

quenchers of singlet oxygen than are gases, by several orders of magnitude. Therefore, while greater surface area provides more sites for production of singlet oxygen, it also provides greater quenching. It should be possible to improve the efficiency of these sensitizers by optimizing such factors.

Covalently immobilized sensitizers did not appear to offer any advantage over the more easily prepared physically adsorbed preparations, as long as the sensitizer loading was kept low (2.5 mg/g of support). At high sensitizer loadings (25 mg/g of support) singlet oxygen production decreased dramatically. When powdered Rose Bengal was applied directly onto the rubber cement and used as the sensitizer, singlet oxygen production was also very low. Rose Bengal, physically adsorbed at low loading (3 μ/cm^2) directly onto the surface of the glass plate by evaporation of a methanol or acetone solution, was not a good sensitizer; Methylene Blue similarly prepared was even poorer.

While the photosensitizing efficiency of Methylene Blue immobilized on alumina was relatively high, these preparations bleached more rapidly than the others and were generally useless after only 1 or 2 h of illumination. Some bleaching occurred with all the preparations except Sensitox. Sensitox I was not bleached during illumination and was a moderately efficient sensitizer although Rose Bengal immobilized on silica gel or glass beads was much better. Plates prepared with Sensitox I could be reused with only slight reductions in rate of singlet oxygen production even after several hours of illumination. Sensitox II, on the other hand, is one of the poorest sensitizers under these conditions.

Sensitox I and II differ in the structure of the polymer support to which the Rose Bengal is attached. Sensitox I is composed of Rose Bengal covalently bound to a hydrophobic polymer and is designed for use in organic solvents. Sensitox II consists of Rose Bengal bound to a more hydrophilic polymer and is designed for use in aqueous solution. The greater efficiency of Sensitox I may be due to the greater ease with which oxygen can penetrate the more hydrophobic Sensitox I polymer.

This singlet oxygen generating system was used to measure the relative rates of singlet oxygen oxidation of deoxyguanosine and 2,5-bis(hydroxymethyl)furan in pH 10 sodium carbonate and of L-histidine and L-tryptophan in water/methanol, 50:50 (v/v). The results are presented in Table III. The value of the ratio of the rate of singlet oxygen oxidation of histidine to that of tryptophan was found to be 0.65 ± 0.15 and is in fair agreement with the value of 0.57 found by Nilsson, Merkel, and Kearns.[65] The rate ratio obtained by using 2,5-bis(hydroxymethyl)furan and deoxyguanosine was used in a more complete kinetic analysis of photooxidation of deoxyguanosine to help establish the role of singlet oxygen in such reactions. The rate ratio obtained agrees with the minimum rate ratio obtained at pH 10 by using dyes in solution, as would be required if the reaction only involved singlet oxygen in this system.[66]

Summary

The method described here is a simple and yet sound way to generate singlet oxygen for use in kinetic analysis of singlet oxygen oxidations of reactive substrates. Because none of the systems tested so far are very efficient at generating singlet oxygen, only those substrates which react relatively rapidly can be studied. Optimization of singlet oxygen yields should make this system generally applicable for study of kinetics of singlet oxygen reactions in aqueous as well as organic solvents.

The distance dependence of the oxidation rate corresponds roughly to that expected for a reaction involving singlet oxygen as an intermediate. The rate of superoxide formation was found to be less than 1% of the rate of substrate oxidations, indicating that superoxide is not involved in the observed reactions.

Several immobilized sensitizer preparations were tested for their efficiency in singlet oxygen production. Immobilization of sensitizers by physical adsorption was found to be as useful as covalent attachment. Because of resistance to sensitizer bleaching, Sensitox I coated plates were found to be most suitable for long illuminations although this sensitizer was not the most efficient source of singlet oxygen.

This system was used to measure relative rates of singlet oxygen oxidation of two pairs of substrates. The relative rates of singlet oxygen oxidation of histidine and tryptophan were found to agree with the previously reported value.

The singlet oxygen generating system described here should be useful not only for kinetic studies but also for examining some of the biological effects of singlet oxygen since it provides a source of singlet oxygen that lacks many of the disadvantages that complicate interpretation of biochemical data. Some aspects of these applications of this technique are under investigation in this laboratory.

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Registry No. Oxygen, 7782-44-7.

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(65) Nilsson, R.; Merkel, P. B.; Kearns, D. R. *Photochem. Photobiol.* **1972**, *16*, 117-124.

(66) Midden, W. R.; Wang, S. Y., manuscript in preparation.