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Detection of photolytically produced hydroxyl radicals by reaction with 2,2,2-trichloroethanol

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Summary

A screening technique for the detection of the hydroxyl radical on photolysis of drugs or other chemicals is described. The technique depends upon scavenging the radical with 2,2,2-trichloroethanol in buffered aqueous solution and determining the liberated chloride ion. The system was validated using sodium nitrite as a model source of photolytically produced hydroxyl radicals. Competition kinetic experiments were performed using glucose, glycine and sulphate ion as competitive scavengers. The drugs metronidazole, phenylephrine and chloroquine diphosphate were examined for hydroxyl radical production on simulated sunlight photolysis, metronidazole caused no chloride liberation from 2,2,2-trichloroethanol on the time scale studied. In the case of chloroquine diphosphate, chloride ion liberated directly from the drug on its photolysis interfered with kinetic evaluation of the results. Chloride ion production from 2,2,2-trichloroethanol induced by phenylephrine was kinetically equivalent to that caused by the hydroxyl radical.

Introduction

The hydroxyl radical ($OH^{\bullet}$) is implicated in the phototoxicity of certain drugs and other chemicals (Halliwel and Gutteridge, 1985) and also is of potential importance in the light stability of drug formulations (Barnes and Sugden, 1986). In the presence of molecular oxygen, hydroxyl radical formation via the superoxide anion is possible:

$$\text{Drug} + O_2 \rightarrow (\text{Drug})^{\bullet +} + O_2^{\bullet -}$$

$$2\,O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

The hydroxyl radical can then be produced by photolysis of hydrogen peroxide or by its subsequent reaction with metal ions:

$$H_2O_2 - h\nu \rightarrow 2\,OH^{\bullet}$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

Hydroxyl radical production is also possible in anaerobic conditions. For instance anthraquinones produce the hydroxyl radical on light irradiation by electron transfer to the hydroxyl ion (Bruce, 1974). *p*-Benzoquinone (BQ) forms the hydroxyl radical as follows (Beck and Brus, 1982):

$$BQ + H_2O - h\nu \rightarrow BQH^{\bullet} + OH^{\bullet}$$

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Electron spin resonance spectroscopy is widely used for investigating free radical formation, but the hydroxyl radical is so short-lived that it can only be detected by spin trapping; 5,5-dimethyl-1-pyrroline-*N*-oxide and 2-dimethyl-2-nitrosopropane spin traps have been used to study superoxide-dependent hydroxyl radical production (Decuyper et al., 1983, Motten et al., 1985).

Chemical means of detecting the hydroxyl radical involve allowing the radical to react with a scavenger and measuring either loss of the scavenger or production of a scavenger oxidation product. Most of the work in this area has been concerned with the determination of the hydroxyl radical in biological systems. Methods include the production of ethylene from methional, formaldehyde from dimethylsulphoxide and carbon dioxide from benzoate (Halliwell and Gutteridge, 1985).

Competition kinetics are potentially of use in providing evidence of the identity of the species attacking the scavenger. The degree of quenching of the reaction by the addition of a competitive scavenger depends on the ratio of the rate constants of the reactions between the attacking species and the two scavengers. This method has been used for identifying the formation of singlet oxygen from Rose bengal (Higgins et al., 1968). Competition kinetics have been used in a qualitative manner to identify hydroxyl radicals produced in biological systems (Halliwell and Gutteridge, 1985).

We wish to report the use of 2,2,2-trichloroethanol as a scavenger for detecting the hydroxyl radical by determination of liberated chloride ion.

Materials and Methods

2,2,2-Trichloroethanol (B.D.H., Poole, U.K) was purified by distillation under reduced pressure. Care was taken to avoid contact between the distillate and the vacuum grease used to lubricate the ground-glass joints of the condenser, so as to avoid a pink discolouration of the 2,2,2-trichloroethanol. A 0.1 M aqueous solution of the purified material contained typically 0.3 parts per million (p.p.m.) chloride ion.

Phenylephrine free base was prepared from a solution of phenylephrine hydrochloride (2 g) in water (10 ml) by the addition of ammonia solution 35% w/v (0.1 ml). The solution was cooled to 4° C and the precipitated free base washed with water at 4° C and dried under vacuum at 40° C for 5 hs; m.p. 168–169° C (Lit. 169–172° C) (Windholz 1983). The chloride ion content of a 5×10^{-3} M aqueous solution of the product was 0.5 p.p.m.

All other materials were of the highest commercially available grade and were used without further purification.

Methods

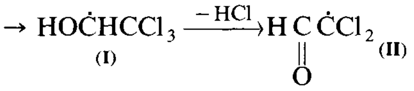
Chloride ion was determined by the method of Florence and Farrar (1971), i.e. to 20 ml of sample in a 25 ml volumetric flask was added saturated ethanolic mercuric thiocyanate solution (2 ml) and 15.1% ferric nitrate nonahydrate in 35% m/v aqueous perchloric acid (2 ml); the solution was made up to volume with water and the absorbance was measured in 4-cm pathlength cells at 460 mm against reagent blank after 5 min or at 5 min ± 5 s for solutions containing sodium nitrite. Chloride ion concentrations were obtained by comparison with a standard calibration curve, prepared daily. For solutions containing sulphate ion, chloride ion concentrations were determined by comparison with standard solutions containing an equivalent concentration of sulphate ion. This procedure was also adopted for experiments with chloroquine diphosphate where matching concentrations were added to the calibration standards.

Photolysis was carried out with 'Sylvania' blacklight-blue fluorescent lights in the apparatus of Evans et al. (1975), using up to 6 stoppered 50 ml Nessler cylinders mounted in a rotating carrousel within the apparatus. Sample solutions (25 ml) contained 1 M 2,2,2-trichloroethanol, 0.03 M borate buffer pH 7.0 and either 8.7×10^{-4} M sodium nitrite or various concentrations of metronidazole, phenylephrine or chloroquine diphosphate. Photolysis times were 20 min for samples containing sodium nitrite and 60 min for samples containing metronidazole, phenylephrine or chloroquine diphosphate. When competition kinetic experiments were performed, conditions were as above except that 0.1 M 2,2,2-trichloroethanol was replaced without various concentrations of that compound with glucose, glycine or sulphate ions added.

Results and Discussion

The two abstractable hydrogen atoms of 2,2,2-trichloroethanol are equivalent and attack by the hydroxyl radical on either could be expected to lead to the liberation of chloride ion, by analogy with other chlorohydrins such as chloral hydrate (Buley et al., 1966). The likely mechanism is:

$$HOCH_2CCl_3 + OH^{\cdot}$$



$$\rightarrow \underset{(I)}{HO\dot{C}HCCl_3} \xrightarrow{-HCl} \underset{(II)}{H\underset{\underset{O}{\|}}{C}\dot{C}Cl_2}$$

Chloral hydrate was found to be unsuitable as a scavenger: adjustment of the pH of a 0.1 M solution of chloral hydrate to pH 7.0 led to the liberation of chloride ion by hydrolysis. 2,2,2-Trichloroethanol was stable in this respect over the time scale of the experiments. Chloride ion was determined by the spectroscopic method of Florence and Farrar (1971), which in turn is based on earlier work (Iwasaki et al., 1952). The reactions involved can be depicted as:

$$2\ Cl^- + Hg(SCN)_2 \rightleftharpoons HgCl_2 + 2\ SCN^-$$

$$4\ Cl^- + Hg(SCN)_2 \rightleftharpoons HgCl_4^{2-} + 2\ SCN^-$$

$$SCN^- + Fe^{3+} \rightleftharpoons Fe(SCN)^{2+}$$

Determination of chloride in solutions containing 8.7×10^{-4} M sodium nitrite resulted in a sample solution which exhibited a linear increase in absorbance with time (0.00475 absorbance units/min, $r = 0.9978$). The rate of absorbance increase was constant on a day to day basis and was not affected by the presence of any other constituents in the sample solution. It has been reported that nitrite ion will displace chloride ion from a mercuric chloride complex (Aylett, 1973). Hence the mechanism of nitrite interference is probably by displacement of thiocyanate ion:

$$2\ NO_2^- + Hg(SCN)_2 \rightarrow Hg(NO_2)_3 + 2\ SCN^-$$

This reaction would be expected to be second order with respect to the nitrite ion and mercuric thiocyanate concentration. However, over the relatively short time scale studied the reaction appeared from graphs to be zero order. In the presence of nitrite, the method was unsuitable for determining absolute concentrations of chloride. However, by recording absorbances at 5 min ± 5 s after the addition of the mercuric thiocyanate reagent, drift due to nitrite could be corrected, thus allowing changes in chloride concentration to be accurately determined. This facility was adequate for the present work.

Photolysis of 2,2,2-trichloroethanol alone led only to a negligible increase in chloride ion concentration (Table 1), whereas the addition of sodium nitrite produced a substantial rise.

It was considered desirable to buffer the solutions during photolysis to remove any effects due to gross changes in pH. Borate buffer was chosen on the basis of its likelihood to react with the hydroxyl radical at a slower rate than organic materials. A concentration of 0.03 M borate buffer pH 7.0 was found suitable for buffering solutions during photolysis (Table 2). There was no clear

TABLE 1

Photolysis of 2,2,2-trichloroethanol; effect of sodium nitrite

Sample	Increase in chloride ion concentration (p.p.m.) [a]			
	Replicates			Mean
0.1 M 2,2,2-trichloroethanol	0.05	0.04	0.06	0.05
0.1 M 2,2,2-trichloroethanol + 8.7×10^{-4} M sodium nitrite	2.10	2.19	2.06	2.12
0.1 M 2,2,2-trichloroethanol + 8.7×10^{-4} M sodium nitrite (diffuse light)	0.08	0.04	0.05	0.06

[a] Analysis of variance $F = 2571.57$ ($P < 0.001$)

TABLE 2

Effect of buffer concentration on photolytic chloride ion production from 2,2,2-trichloroethanol + 8.7×10^{-4} M sodium nitrite

Concentration borate buffer pH 7.0	pH after photolysis	Increase in chloride ion concentration
0.005	4.48	1.65
0.010	5.72	1.40
0.015	6.58	1.36
0.020	6.78	1.45
0.025	6.82	1.60
0.030	6.95	1.42

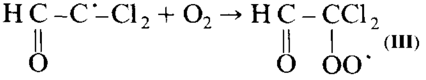
correlation between buffer concentration and chloride production. The rate constants for the reaction between OH$^{\cdot}$ and boric acid or its ionised form $B(OH)_4^-$ have not been determined, but it is likely that they would be similar to be the values for phosphoric acid and dihydrogen phosphate ion. These values, 2.6×10^6 and 2.2×10^6 $M^{-1}s^{-1}$ respectively, are several orders of magnitude lower than would be the case for the reaction between OH$^{\cdot}$ and organic molecules (Ross and Ross, 1977), thus explaining the lack of effect of borate buffer concentration of chloride formation. The photolysis system was insensitive to pH in the range 6.5–7.0 (Table 3). Chloride ion production was linear on the time scale studied. Under anaerobic conditions, following bubbling of the samples with nitrogen, the rate of chloride production was 0.095 p.p.m./minute and under aerobic conditions the rate was higher, at 0.148 p.p.m./minute.

TABLE 3

Effect of pH on photolytic chloride ion production from 2,2,2-trichloroethanol + 8.7×10^{-4} M sodium nitrite

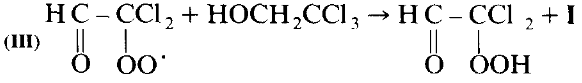
pH		Increase in chloride ion concentration (p.p.m.)
Before photolysis	After photolysis	
7.05	6.91	1.25
6.95	6.70	0.960
6.80	6.88	1.08
6.69	6.56	0.960
6.51	6.38	0.955

In the presence of oxygen, radical **II** would react as follows:



$$\underset{\underset{O}{\|}}{HC}-\dot{C}-Cl_2+O_2\rightarrow\underset{\underset{O}{\|}}{HC}-\underset{\underset{OO^{\cdot}}{|}}{CCl_2}\quad\textbf{(III)}$$

Peroxy radicals of type **III** are capable of abstracting hydrogen atoms from organic molecules (Sheldon 1983) and would react with 2,2,2-trichloroethanol to yield radical **I**, resulting in increased chloride ion production:



$$\textbf{(III)}\ \underset{\underset{O}{\|}}{HC}-\underset{\underset{OO^{\cdot}}{|}}{CCl_2}+HOCH_2CCl_3\rightarrow\underset{\underset{O}{\|}}{HC}-\underset{\underset{OOH}{|}}{CCl_2}+\mathbf{I}$$

Consumption of nitrite ion during photolysis might be expected to lead to a decrease in the rate of chloride formation. This was not the case and may indicate that nitrite ion was regenerated after photolysis. There is supporting evidence that this may be the case obtained from studies on the photolysis of nitrite ion in sea water (Zafiriou, 1974).

The precision of the technique, tested using nitrite ion as a model source of OH$^{\cdot}$, was satisfactory (Table 4).

Any radical capable of abstracting hydrogen atoms from 2,2,2-trichloroethanol will cause chloride ion formation. Thus specificity to the hy-

TABLE 4

Precision of the 2,2,2-trichloroethanol method of hydroxyl radical estimation

	Absorbance at 460 nm [a]	
	After photolysis [b]	Increase
	0.438	0.370
	0.417	0.349
	0.452	0.384
	0.461	0.393
	0.424	0.356
	0.470	0.402
Number of replicates	6	6
Mean	0.444	0.376
Standard deviation	0.0209	0.0209
Coefficient of variation	4.72%	5.58%

All replicate solutions contained 8.7×10^{-4} M sodium nitrite

[a] Florence and Farrar, 1971.

[b] Absorbance before photolysis 0.068.

droxyl radical cannot be attained on this or any other system relying on the abstraction of hydrogen atoms from an aliphatic compound, by chemical means alone. Previous workers have used aromatic compounds such as nitrobenzene as hydroxyl radical scavengers and used the ratio of ortho-, meta- and para-hydroxylated products to characterise the hydroxyl radical. However, the isomer ratio has been found to vary depending on the technique used to generate the radical (Floyd et al., 1984).

The use of competition kinetics was considered appropriate to render the photolytic technique specific to $OH^{\cdot}$. The rate constants for the reaction of the hydroxyl radical with other organic compounds will be specific to that radical. Absolute rate constants cannot be measured without specialist equipment, but relative rate constants, with two competing substrates, can be conveniently determined. The kinetics of the reaction of a reactive species with two competing substrates has been summarised (Lewis, 1978). The competition between 2,2,2-trichloroethanol and a competing scavenger can be set out thus:

$OH^{\cdot} + T - k_T \rightarrow Cl^- +$ other products

$OH^{\cdot} + X - k_X \rightarrow Y$

T = 2,2,2-Trichloroethanol, X = competing scavenger, Y = products, k_T = second order rate constant for the reaction between T and $OH^{\cdot}$, k_X = second order rate constant for the reaction between X and $OH^{\cdot}$. The rate equations for these reactions are:

$$\frac{d[Cl^-]}{dt} = -\frac{d[T]}{dt} = k_T[OH^{\cdot}][T] \qquad (1)$$

$$\frac{d[Y]}{dt} = -\frac{d[X]}{dt} = k_X[OH^{\cdot}][X] \qquad (2)$$

since it is reasonable to assume that the reactions are first order with respect to $OH^{\cdot}$, T and X

$$\frac{d[Cl^-]}{d[Y]} = \frac{k_T[OH^{\cdot}][T]}{k_X[OH][X]} = \frac{k_T[T]}{k_X[X]} \qquad (1)/(2)$$

If [T] and [X] do not change in the course of the experiment then

$$\frac{[Cl^-]}{[Y]} = \frac{k_T[T]}{k_X[X]} \qquad (3)$$

$[Y] = [Cl^-]_o - [Cl^-]$ where $[Cl^-]_o$ = chloride ion concentration in the absence of the second scavenger.
Thus

$$\frac{[Cl^-]}{[Cl]_o - [Cl]} = \frac{k_T[T]}{k_X[X]}$$

Therefore

$$\frac{A}{A_0 - A} = \frac{k_R[T]}{[X]}$$

Where A and A_0 are the absorbances produced on measuring the chloride ion concentration, k_R = the relative rate constant k_T/k_X. Eq. (3) can be transcribed as

$$\frac{A_0}{A} = 1 + k_R\frac{[X]}{[T]} \qquad (4)$$

and

$$\frac{1}{A} = \frac{1}{A_0}\left[1 + k_R\frac{[X]}{[T]}\right] \qquad (5)$$

Eqn. (4) shows that errors in measuring A_0 will bias the slope of the graph and influence k_R, but the use of Eq. (5) will lead to a more accurate estimate of A_0 than could be obtained with the small number of replicates possible in each experiment. The values of A_0 obtained using Eqn. (5) were subsequently used in Eqn. (4) to obtain a more accurate estimate of k_R. Eqn. (5) was not used for yielding values of k_R directly since it does not allow data from replicate experiments to be easily pooled.

The competing scavengers were selected on the basis of their light transparency at more than 300 nm and their good aqueous solubility.

TABLE 5

Comparison of experimentally determined estimates of k_R with values derived from the literature (Ross and Ross, 1977)

Scavenger	k_X $M^{-1}s^{-1} \times 10^6$	k_T $M^{-1}s^{-1} \times 10^6$	k_R Literature range	k_R Experimentally determined [c]
Glucose	380 –2 200	1 130 ($n = 4$)	3.4 –20.2	3.20 (air [b]) 1.20 (N_2 [b])
Glycine (Zwitterion)	5.6– 260	60.6 ($n = 5$)	0.0422– 2.39	0.784 (air) 0.322 (N_2)

[a] Assumes that $k_T = 1.09 \times 10^8$ $M^{-1}s^{-1}$

[b] Standard concentration range.

[c] Mean of two determinations.

TABLE 6

Competition kinetics: glucose as scavenger (standard concentration range)

Condition	[X] (M × 10^{-2}) [a]	[T] (M × 10^{-2}) [a]	A	$1/A$	A_0 [b]	[X]/[T]	A_0/A	k_R [c]
Aerobic	8.33	1.67	0.032	31.25		5.00	16.96	
	6.67	3.33	0.074	13.51		2.00	7.33	
	5.00	5.00	0.122	8.20	0.543	1.00	4.45	3.20
	4.17	5.83	0.150	6.67		0.71	3.62	
	0.00	10.00	0.685	1.46		0.00	0.79	
	0.00	10.00	0.700	1.43		0.00	0.78	
Anaerobic	8.33	1.67	0.050	20.00		5.00	7.03	
	6.67	3.33	0.111	9.01		2.00	3.17	
	5.00	5.00	0.152	6.58		1.00	2.31	
	4.17	5.83	0.162	6.17	0.352	0.71	2.17	1.20
	0.00	10.00	0.390	2.56		0.00	0.90	
	0.00	10.00	0.399	2.51		0.00	0.88	

[a] X = glucose; T = 2,2,2-trichloroethanol.

[b] Evaluated by least-squares analysis from Eq. 5.

[c] Relative reaction rate constant (k_X/k_t) evaluated by least-squares analysis from Eqn. 4.

TABLE 7

Competition kinetics: glucose as scavenger (low concentration range)

Condition	[X] (M × 10^{-2}) [a]	[T] (M × 10^{-2}) [a]	A	$1/A$	A_0 [b]	[X]/[T]	A_0/A	k_R [b]
Aerobic	1.667	8.33	0.216	4.63		0.200	2.39	
	1.000	9.00	0.283	3.53		0.111	1.83	
	0.667	9.33	0.322	3.11		0.071	1.61	
	0.333	9.67	0.404	2.48	0.517	0.034	1.28	7.23
	0.000	10.00	0.534	1.87		0.000	0.97	
	0.000	10.00	0.553	1.81		0.000	0.94	
Anaerobic	1.000	10.00	0.297	3.37		0.100	1.34	
	0.600	10.00	0.314	3.18		0.060	1.27	
	0.400	10.00	0.346	2.89		0.040	1.15	
	0.300	10.00	0.364	2.75	0.398	0.030	1.09	3.67
	0.000	10.00	0.378	2.65		0.000	1.05	
	0.000	10.00	0.426	2.35		0.000	0.93	

[a] X = glucose, T = 2,2,2-trichloroethanol.

[b] Evaluated as in Table 6.

TABLE 8

Competition kinetics: glycine as scavenger

Condition	[X] (M × 10^{-2}) [a]	[T] (M × 10^{-2}) [a]	A	$1/A$	A_0 [b]	[X]/[T]	A_0/A	k_R [b]
First replicate								
Aerobic	8.33	1.67	0.111	9.01		5.00	5.38	
	6.67	3.33	0.215	4.65		2.00	2.78	
	5.00	5.00	0.313	3.20		1.00	1.91	
	4.17	5.83	0.376	2.66	0.597	0.71	1.59	0.878
	0.00	10.00	0.600	1.67		0.00	1.00	
Anaerobic	8.33	1.67	0.186	5.38		5.00	2.57	
	6.67	3.33	0.342	2.92		2.00	1.40	
	5.00	5.00	0.371	2.70		1.00	1.29	
	4.17	5.83	0.406	2.46	0.477	0.71	1.18	0.296
	0.00	10.00	0.448	2.23		0.00	1.06	
	0.00	10.00	0.437	2.29		0.00	1.09	
Second replicate								
Aerobic	8.33	1.67	0.513	6.54		5.00	4.43	
	6.67	3.33	0.280	3.57		2.00	2.42	
	5.00	5.00	0.407	2.46		1.00	1.67	
	4.17	5.83	0.462	2.16	0.678	0.71	1.47	0.689
	0.00	10.00	0.687	1.46		0.00	0.99	
	0.00	10.00	0.663	1.51		0.00	1.02	
Anaerobic	8.33	1.67	0.182	5.50		5.00	2.85	
	6.67	3.33	0.327	3.06		2.00	1.59	
	5.00	5.00	0.441	2.27		1.00	1.18	
	4.17	5.83	0.455	2.20	0.519	0.71	1.14	0.348
	0.00	10.00	0.446	2.24		0.00	1.16	
	0.00	10.00	0.441	2.27		0.00	1.18	

[a] X = glycine, T = 2,2,2-trichloroethanol.

[b] Evaluated as in Table 6.

Glucose was chosen as a scavenger with high reaction rate with $OH^{\bullet}$ and glycine as one with a relatively low reaction rate (Table 5).

The intraexperimental variability of the competition experiments was low, as indicated by the high correlation coefficients obtained (Tables 6–8). Over the range studied, the regressions were linear, supporting the assumptions made in the derivation of Eqs. (4) and (5). The much larger values of k_R obtained under anaerobic conditions can be explained by consideration of the interactions which are possible during photolysis. In the case of glucose (TH = 2,2,2-trichloroethanol, GH-glucose, $R^{\bullet}$ = dehydrohalogenated radical produced from 2,2,2-trichloroethanol):

$$TH + OH^{\bullet} \rightarrow R^{\bullet} + Cl^{-} + H_2O \qquad (6)$$

$$GH + OH^{\bullet} \rightarrow G^{\bullet} + H_2O \qquad (7)$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \qquad (8)$$

$$G^{\bullet} + O_2 \rightarrow GOO^{\bullet} \qquad (9)$$

$$TH + ROO^{\bullet} \rightarrow R^{\bullet} + ROOH + Cl^{-} \qquad (10)$$

$$Th + GOO^{\bullet} \rightarrow R^{\bullet} + GOOH + Cl^{-} \qquad (11)$$

$$GH + ROO^{\bullet} \rightarrow G^{\bullet} + ROOH \qquad (12)$$

$$GH + GOO^{\bullet} \rightarrow G^{\bullet} + GOOH \qquad (13)$$

The hydroxyl radical can react with either 2,2,2-trichloroethanol or glucose (Eqs. 6 and 7). In the presence of oxygen the radicals thus generated can yield peroxy radicals (Eqs. 8 and 9) which can undergo further hydrogen abstraction reactions leading to chloride ion production (Eqs. 10 and 11) or to the formation of products other than chloride ion (Eqns. 12 and 13). Other reactions, such as decomposition of hydroperoxides or radical–radical dimerisations can be disregarded. The higher values of k_R seen in the aerobic experiments would be caused by reduced chloride ion formation for a given concentration of scavenger compared to the theoretical amount. This would be so if the rate constants for reactions 10 and 11 were lower than those for reactions 12 and 13. Steric hindrance due to the bulky CCl_3 group of 2,2,2-trichloroethanol would account for this. Consequently more accurate estimates of k_R should be obtained from the anearobic experiments than from the aerobic ones. This is difficult to establish since there are no published values for the rate of reaction between $OH^\cdot$ with 2,2,2-trichloroethanol. Indirect comparisons can, however, be made by noting that the rate of reaction of $OH^\cdot$ with 2,2,2-trichloroethanol as being 0.475 times that of the reaction of this radical with Fe^{2+} (Walling 1975). The literature estimate of the rate of reaction between $OH^\cdot$ and Fe^{2+} is 2.3×10^8 $M^{-1}s^{-1}$ (Ross and Ross, 1977). Thus it is possible to estimate the rate of reaction between $OH^\cdot$ and 2,2,2-trichloroethanol as being 1.09×10^8 $M^{-1}s^{-1}$. This figure was used to calculate the values of k_R from reaction rate constants obtained from the literature (Table 5). These values had a wide range and it was not possible to correlate the estimates of k_R obtained in this work with those of the literature.

Repeating the glucose competition experiments with a lower concentration of the scavenger (Table 7) gave higher estimates of k_R for both aerobic and anaerobic conditions than did the higher concentration range (Table 6). Although these differences could be due to interexperimental variation, it may be that in the case of the higher glucose concentration range, the regression line was curved near the origin leading to an apparently higher value of k_R for the lower concentration range.

In the case of glycine (Table 8) replicate experiments showed that there was a considerable interexperimental variability which may have been due to error in measuring A_0.

Sulphate does not form peroxides in the same way that glucose and glycine can, so it was used as a scavenger to reduce complicating factors from reactions such as 10–13.

Although sulphate ion interferes with chloride determination by complexing with Fe^{3+} and reducing the absorbance of the resulting sample solution, the interference was overcome by incorporating matching concentrations of sulphate into the standard chloride solutions used for quantification.

By analogy with oxyanions such as phosphate, sulphate is likely to react with the hydroxyl radical by electron transfer:

$$SO_4^{2-} + OH^\cdot \rightarrow SO_4^{\cdot-} + OH^-$$

Competition kinetic studies using sulphate as the competitive scavenger with experiments in both aerobic and anaerobic conditions carried out in duplicate showed that the estimate of k_R obtained under aerobic conditions was significantly greater ($P < 0.05$, *t*-test; Zar, 1979; Table 9) than the corresponding anaerobic value. However, the difference between the two conditions was much less than in the case of glucose or glycine.

The reaction of SO_4^{2-} with the hydroxyl radical is likely to yield the sulphate radical anion $SO_4^{\cdot-}$, which is capable of abstracting hydrogen atoms from aliphatic compounds. The anion radical can also dimerise to form the persulphate ion $S_2O_8^{2-}$ and this reaction proceeds 10 × faster than that of hydrogen abstraction from methanol or ethanol (Dogliotti and Hayon, 1967). Thus, in the present work, the probability of the sulphate radical anion causing hydrogen abstraction from 2,2,2-trichloroethanol and hence Cl^- formation is small.

The values of k_R obtained in the present work gave the rank order of reaction rate constants of the hydroxyl radical with the hydroxyl scavengers as glucose > 2,2,2-trichloroethanol > glycine > sulphate ion. This agrees with available reaction rate data (Table 5) and with theoretical considerations.

Three diverse drugs were chose for screening for their ability to generate the hydroxyl radical on photolysis. Metronidazole did not cause a rise in chloride concentration over the time scale of the experiment (Table 10) in either aerobic or anaerobic conditions. It was concluded that this drug did not produce the hydroxyl radical under the conditions studied.

TABLE 9

Competition kinetics: sulphate as scavenger

Condition	[X] (M × 10^{-2}) [a]	[T] (M × 10^{-2}) [a]	A [b]	A [c]	$1/A$ [c]	A_0 [d]	[X]/[T]	A_0/A	k_R [d]
First replicate									
Aerobic	6.98	1.67	0.180	0.217	4.60		4.19	2.95	
	5.59	3.33	0.317	0.368	2.72		1.68	1.74	
	4.19	5.00	0.404	0.451	2.22		0.84	1.42	
	3.49	5.83	0.448	0.490	2.04	0.641	0.60	1.13	0.465
	0.00	10.00	0.669	0.669	1.49		0.00	0.96	
	0.00	10.00	0.632	0.632	1.58		0.00	1.01	
Anaerobic	5.59	3.33	0.200	0.233	4.30		1.68	1.75	
	4.19	5.00	0.266	0.297	3.37		0.84	1.37	
	3.49	5.83	0.294	0.322	3.11	0.406	0.60	1.26	0.443
	0.00	10.00	0.415	0.415	2.41		0.00	0.98	
	0.00	10.00	0.396	0.396	2.53		0.00	1.03	
Second replicate									
Aerobic	6.98	1.67	0.109	0.132	7.59		4.19	2.91	
	5.59	3.33	0.186	0.216	4.63		1.68	1.78	
	4.19	5.00	0.243	0.271	3.69	0.384	0.84	1.41	0.458
	3.49	5.83	0.273	0.298	3.35		0.60	1.28	
	0.00	10.00	0.381	0.381	2.62		0.00	1.01	
	0.00	10.00	0.401	0.401	2.49		0.00	0.96	
Anaerobic	6.98	1.67	0.135	0.164	6.11		4.19	2.80	
	5.59	3.33	0.222	0.256	3.90		1.68	1.79	
	4.19	5.00	0.310	0.346	2.89	0.458	0.84	1.32	0.435
	3.49	5.83	0.319	0.349	2.86		0.60	1.31	
	0.00	10.00	0.486	0.486	2.06		0.00	0.94	
	0.00	10.00	0.452	0.452	2.21		0.00	1.01	

[a] X = SO_4^{2-}, T = 2,2,2-trichloroethanol.

[b] Uncorrected for the presence of SO_4^{2-}.

[c] Corrected for the presence of SO_4^{2-}.

[d] Evaluated as for Table 6.

TABLE 10

Effect of test drugs on the production of chloride ion from 2,2,2-trichloroethanol

Drug	Concentration (M)	Increase in chloride ion concentration		
		Anaerobic (mean)	Aerobic (mean)	Normalised aerobic mean [a]
Metronidazole	5×10^{-5}	0.137, 0.120 (0.128)	0.116, 0.103 (0.110)	0.074
	5×10^{-4}	0.103, 0.107 (0.105)	0.112, 0.112 (0.112)	0.075
	5×10^{-3}	0.090, 0.090 (0.090)	0.077, 0.099 (0.088)	0.059
Phenylephrine	5×10^{-5}	0.080, 0.080 (0.080)	0.060, 0.078 (0.069)	0.046
	5×10^{-4}	0.130, 0.113 (0.122)	0.133, 0.137 (0.135)	0.091
	5×10^{-3}	2.20, 2.18 (2.19)	2.37 , 244 (2.40)	1.61
Chloroquine diphosphate	5×10^{-5}	3.03, 2.77 (2.90)	1.61 , 1.61 (1.61)	1.08
	5×10^{-4}	2.17, 1.98 (2.08)	0.945, 0.906 (0.926)	0.623
	5×10^{-3}	0.907, 1.025 (0.966)	0.584, 0.603 (0.594)	0.399

[a] As the 2,2,2-trichloroethanol is 1.49 times more sensitive in aerobic conditions these values have been reduced by the corresponding factor to make allowance.

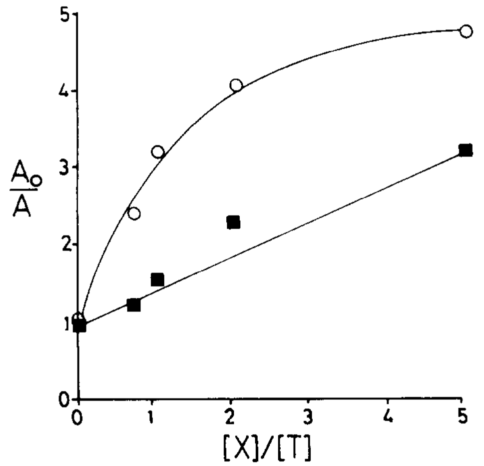


Fig. 1. Sulphate ion competition kinetic experiments; chloride ion production caused by phenylephrine (■) and chloroquine diphosphate (○).

Both phenylephrine and chloroquine diphosphate did cause production of chloride in both aerobic and anaerobic conditions. With phenylephrine, competition studies with single concentrations of organic scavengers gave the same rank order of k_R values as was found with the nitrite ion model system (Table 10).

More comprehensive competition studies using the sulphate ion as scavenger under aerobic conditions showed that phenylephrine gave a value of k_R which was not significantly different from that obtained in the nitrite model system (pooled aerobic results $F = 0.15$, $F_{0.05(1)1,14} = 4.35$ (Zar, 1974); Fig. 1, Table 11).

The formation of the hydroxyl radical on photoirradiation of phenylephrine has previously been proposed by Al Taii et al. (1982). Although confirmation with an independent technique, such as electron spin resonance, would be required to obtain definite proof of hydroxyl radical production from phenylephrine, photoionisation of the drug could occur resulting in superoxide formation in aerobic conditions (Fig. 2). This in turn could lead to $OH^\cdot$ formation. The significance of $OH^\cdot$ formation on the photostability of phenylephrine in solution remains to be established.

TABLE 11

Kinetic investigation of test drugs: sulphate as scavenger

Drug	[X] ($M \times 10^{-2}$) [a]	[T] ($M \times 10^{-2}$) [a]	A [b]	A [c]	$1/A$ [c]	A_0 [d]	[X]/[T] [a]	A_0/A	k_R [d]
Phenylephrine									
	8.33	1.67	0.126	0.159	6.29		5.00	3.27	
	6.67	3.33	0.184	0.220	4.54		2.00	2.35	
	5.00	5.00	0.287	0.328	3.05		1.00	1.58	
	4.17	5.83	0.369	0.412	2.43	0.519	0.71	1.26	0.456
	0.00	10.00	0.537	0.537	1.86		0.00	0.97	
	0.00	10.00	0.524	0.524	1.91		0.00	0.99	
Chloroquine diphosphate									
	8.33	1.67	0.054	0.068	14.68		5.00	4.82	
	6.67	3.33	0.067	0.080	12.45		2.00	4.09	
	5.00	5.00	0.089	0.102	9.84	0.328 [e]	1.00	3.23	
	4.17	5.83	0.121	0.135	7.41		0.71	2.43	
	0.00	10.00	0.326	0.326	3.07		0.00	1.01	
	0.00	10.00	0.321	0.321	3.12		0.00	1.02	

[a] $X = SO_4^{2-}$, T = 2,2,2-trichloroethanol.

[b] Uncorrected for SO_4^{2-}.

[c] Corrected for SO_4^{2-}.

[d] Evaluated as for Table 6.

[e] Calculated from points [X]/[T] 0, 0.71 and 1.00.

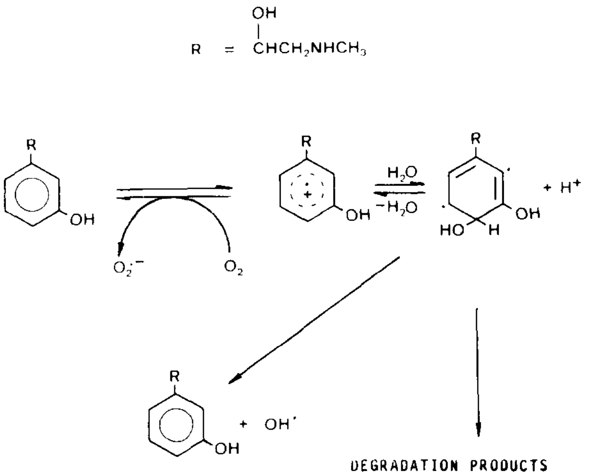


Fig. 2. Possible mechanism of hydroxyl radical production on photolysis of phenylephrine.

In the case of chloroquine diphosphate, the rank order of estimates of k_R obtained with organic scavengers was not the same as found with the nitrite model system (Table 10). Quenching of chloride production by sulphate was non-linear (Table 12, Fig. 1). This could be accounted for by direct production of chloride ion from chloroquine which occurs on photolysis (Moore and Hemmens, 1982) and which would not be quenched by sulphate ion.

Conclusion

Experiments using nitrite ion as a model source of photolytic hydroxyl radicals showed that chloride estimation from 2,2,2-trichloroethanol, combined with competition experiments, is a viable means of studying hydroxyl radical production.

The photolysis of phenylephrine led to the production of reactive intermediates which were capable of abstracting hydrogen atoms from 2,2,2-trichloroethanol. In aerobic conditions the intermediate was kinetically equivalent to the hydroxyl radical.

The technique described in this work has potential application in studying mechanisms of drug photodegradation and phototoxicity. Various authors (Merville et al., 1983; Moore, 1977; Moore and Tamat, 1980; Moore and Hemmens, 1982; Moore et al., 1983) have used in vitro screening techniques to differentiate between Type I (free radical-mediated) and Type II (singlet oxygen-mediated) mechanisms of drug phototoxicity. Type I reactions are commonly detected by monitoring the polymerisation of acrylamide. This technique requires anaerobic conditions and so is unsuitable for studying the effect of oxygen on the free radical process. Monitoring chloride production from 2,2,2-trichloroethanol may be a useful alternative for detecting type I mechanisms.

TABLE 12

Kinetic investigation of test drugs: organic scavengers

Test Drug	A Replicates	A Mean	A_0 Replicates	A_0 Mean	k_R [a]
Phenylephrine					
Glucose	0.148	0.145			4.25
	0.142		0.645		
			0.581	0.613	
Glycine	0.352	0.346			1.78
	0.339				
Chloroquine diphosphate					
Glucose	0.067	0.068			7.93
	0.068		0.286		
			0.293	0.290	
Glycine	0.164	0.166			
	0.168				3.23

[a] Calculated from A_0/A, since $[X]/[T] = 1$.

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