Biochimica et Biophysica Acta, 430 (1976) 209–224
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BBA 47084

THE ONE-ELECTRON TRANSFER REDOX POTENTIALS OF FREE RADICALS

I. THE OXYGEN/SUPEROXIDE SYSTEM

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(Received September 26th, 1975)

SUMMARY

The method of determination of Redox potentials of radicals, using the pulse radiolysis technique, is outlined. The method is based on the determination of equilibrium constants of electron transfer reactions between the radicals and appropriate acceptors. The limitations of this technique are discussed.

The redox potentials of several quinones-semiquinones are calculated, as well as the standard redox potential of the peroxy radical. $E^{0}_{O_2/O_2^-} = -0.33$ V and the redox oxidation properties of the peroxy radical in various systems and pH are discussed. The value determined for the redox potentials of O_2/O_2^- is higher by more than 0.2 V than earlier estimates, which has important implications on the possible role of O_2^- in biological processes of O_2 fixation.

INTRODUCTION

Ever since Michaelis [1] presented his "Theory of the reversible two-step oxidation", attempts have been made to measure redox potentials of each of the single steps leading to an overall two-electron oxidation-reduction.

The shape of the potentiometric titration curve depends on the concentration of the one-electron oxidation-reduction product, the higher its concentration (under equilibrium condition) the better is the separation of the two steps. Analysis of the shape of such curves enable the calculation of the redox potentials of the separate steps provided that the intermediate is relatively stable [1–3]). The systems O_2/H_2O_2 and quinone (Q)/hydroquinone (QH_2) which are dealt with in this study, do not fulfill this condition in aqueous solution at neutral pH.

The O_2^- radical is formed in almost every irradiated solution where O_2 is present [4]. It has also been shown to participate in enzymatic reactions of biological

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Abbreviations: Q, quinone; QH_2, hydroquinone; DMBQ, 2,5 dimethyl-*p*-benzoquinone; NQS, 1,4-naphthoquinone-2-sulfonate.

importance [5]. The redox potential $E^\circ_{O_2/O_2^-}$ has been calculated and many different values are given in the literature: ±0.005 V [6], −0.36 V [7], −0.19 V [8], −0.59 V [9], −0.56 V [10], −0.33 V [11, 13, 14]. Recently, Chevalet et al. [12] determined a value of −0.27 V by polarography. Quinones participate in many biological systems [15] as electron acceptors and many E°_{Q/Q^-} have been evaluated, mostly by polarographic methods in nonaqueous solutions (ref. 16 and references cited therein).

For the values of E°_{Q/Q^-} in neutral aqueous solution there is very limited information available in the literature, owing to the short life-time of the semiquinone radicals. Patel and Wilson [17] have shown the existence of electron transfer equilibrium between O_2^- and some quinones. Pulse radiolysis was recently shown to be a useful technique in measuring one-electron redox potentials [18]. Because of the short life-times of the radicals produced, careful examination must be resumed in order to ensure that thermodynamic equilibrium exists for the systems between which an electron is transferred. The assumption that such an equilibrium exists in several radical systems [19, 20] is now realized to be incorrect (Rao, P. S. and Hayon, E., personal communication). Furthermore, all the redox potentials reported in these studies [19–21] should be critically regarded, since they refer one-electron transfer reactions to two-electron redox potentials. However, the pulse radiolysis technique has already been successfully applied to the measurement of the one-electron redox potentials of several nitro-aromatic and nitro-heterocyclic compounds [22].

In this study we try to establish further the one-electron redox potential for the O_2/O_2^- system and the Q/Q^- system. The implication of the value obtained on several systems in which O_2^- or its acidic form, HO_2, participate in oxidation-reduction reactions are then discussed.

EXPERIMENTAL

The Varian 7715 linear accelerator was used for pulse radiolysis experiments. Solutions were subjected to 0.1–0.5 μs pulses with a 200 mA current of 5 MeV. For spectral measurements the total concentration of radicals was about 7 μM. For the kinetic and equilibrium measurements their concentration was reduced, using 2 mm aluminium foil, to about 0.5 μM. A Spectrosil irradiation cell, 4 cm long, with an optical path of 12.3 cm, was used. A 1P-28 photomultiplier in conjunction with a Bausch and Lomb 1350 grating monochromator constituted the detection system. Appropriate light filters were used to minimize photochemical and scattered light effects. All reagents were of purest available grade and were used without further purification. Solutions were prepared in triply distilled water immediately before irradiation. No change in the spectrum of the quinones due to any probable thermal reaction was observed for at least 2 h after preparation of the solutions. All solutions contained 0.1 M sodium formate except for cases where the formate was replaced by 0.1 M *tert*-butanol and buffered to pH 7.2 using 2 mM phosphate. As none of the pK values of the species considered in our experiments is near 7, no correction is made for partial dissociation. The solutions were saturated by bubbling various mixtures of O_2 and N_2 through the solutions, using the syringe technique. O_2 concentration in solutions under 1 atm of oxygen was assumed to be $1.25 \cdot 10^{-3}$ M or linearly dependent on the partial O_2 pressure otherwise.

Solutions of 10^{-3} M $K_4Fe(CN)_6$ saturated with N_2O were used for dosimetry, assuming $\varepsilon^{420} = 1000\ \mathrm{M^{-1} \cdot cm^{-1}}$ and $G_{\mathrm{Fe(CN)_6^{3-}}} = 5.9$. When using 0.1 formate solution, saturated with O_2, was used as a dosimeter the ε values obtained for the $\mathrm{DMBQ^-}$ (2,5-dimethyl-*p*-benzoquinone) were higher by 4 %.

We have chosen not to work with 2-propanol solutions since the peroxy radicals produced by the reaction of isopropanol radical with O_2 does not necessarily react quantitatively with the quinones.

PROCEDURE FOR CALCULATION OF REDOX POTENTIALS OF RADICALS

The first step in our calculation is to find a reference, one-electron redox potential, to which redox potentials of other free radicals might be referred to in the forthcoming steps. The half-cell reactions relevant to our calculations are the following:

$$\mathrm{Q} + e \rightleftarrows \mathrm{Q^-} \tag{1}$$

$$\mathrm{Q^-} + e \rightleftarrows \mathrm{Q^{2-}} \tag{2}$$

yielding the net two-electron transfer, Reaction 3, for which redox potentials are usually well known:

$$\mathrm{Q} + 2e \rightleftarrows \mathrm{Q^{2-}} \tag{3}$$

Coupled with these oxidation-reduction reactions are the following acid-base equilibria:

$$\mathrm{QH} \rightleftarrows \mathrm{Q^-} + \mathrm{H^+} \tag{1a}$$

$$\mathrm{QH_2} \rightleftarrows \mathrm{QH^-} + \mathrm{H^+} \tag{2a1}$$

$$\mathrm{QH^-} \rightleftarrows \mathrm{Q^{2-}} + \mathrm{H^+} \tag{2a2}$$

For the single steps, 1 and 2, it is obvious that:

$$E^3_{\mathrm{m}_i} = (E^1_{\mathrm{m}_i} + E^2_{\mathrm{m}_i})/2 \tag{I}$$

where ${E_{\mathrm{m}_i}}^1$, ${E_{\mathrm{m}_i}}^2$ and ${E_{\mathrm{m}_i}}^3$ are the half-cell redox potentials of Reactions 1, 2 and 3, respectively, when the ratio of the total concentrations of the oxidized to reduced forms is unity, at pH $= i$, all referred to the normal hydrogen electrode. The IUPAC convention for the potentials is adopted throughout this study.

The dependence of $E^1_{\mathrm{m}_i}$ and $E^2_{\mathrm{m}_i}$ on pH is given by Eqns. II and III:

$$E^1_{\mathrm{m}_i} = E^1_0 + 0.06 \log (K_{1\mathrm{a}} + [\mathrm{H^+}]) \tag{II}$$

$$E^2_{\mathrm{m}_i} = E^2_0 + 0.06 \log \frac{K_{2\mathrm{a}1}\, K_{2\mathrm{a}2} + K_{2\mathrm{a}1}[\mathrm{H^+}] + [\mathrm{H^+}]^2}{K_{1\mathrm{a}} + [\mathrm{H^+}]} \tag{III}$$

${E_0}^j$ is related to the standard potential of the half-cell reaction j, E^{0j}, by Eqns. IIa and IIIa:

$$E^1_0 = E^{01} - 0.06 \log K_{1\mathrm{a}} \tag{IIa}$$

$$E^2_0 = E^{02} - 0.06 \log K_{2\mathrm{a}1} K_{2\mathrm{a}2} / K_{1\mathrm{a}} \tag{IIIa}$$

E^{0j} and $E_0{}^j$ are thus the standard potentials of the half-cells reactions of the fully dissociated and fully protonated species, respectively.

In order to evaluate the single-step redox potentials, we also make use of the semiquinone formation constant, K_4

$$Q+Q^{2-} \rightleftarrows 2Q^- \tag{4}$$

It is convenient to define K'_{4_i}, the apparent semiquinone formation constant, at pH $= i$, by Eqn. IV:

$$K'_{4_i} = \frac{[Q^-]_T^2}{[Q][Q^{2-}]_T} \tag{IV}$$

where

$$[Q^-]_T = [Q^-]+[QH]$$

$$[Q^{2-}]_T = [Q^{2-}]+[QH^-]+[QH_2]$$

K_4 and K'_{4_i} are interrelated by Eqn. V:

$$K_4 = \frac{K_{1a}^2}{K_{2a1}\,K_{2a2}} \cdot \frac{(K_{2a1}\,K_{2a2}+K_{2a1}[H^+]+[H^+]^2)}{(K_{1a}+[H^+])^2} \cdot K'_{4_i} \tag{V}$$

K'_{4_i} is related to the redox potentials of the single steps 1 and 2 by Eqn. VI:

$$E_{m_i}^1-E_{m_i}^2 = 0.06 \log K'_{4_i} \tag{VI}$$

Using Eqns. I and VI, $E_{m_i}{}^1$ and $E_{m_i}{}^2$ may easily be obtained.

In order to get the redox potential of the $O_2/O_2{}^-$ couple, $E_{m_i}{}^5$, the electron transfer equilibrium constant between $O_2{}^-$ and quinones, K_6, may be used

$$O_2+e \rightleftarrows O_2{}^- \tag{5}$$

$$O_2{}^-+Q \rightleftarrows Q^-+O_2 \tag{6}$$

Utilizing Eqn. VII we can thus calculate the redox potential of the single electron-transfer of oxygen provided that K_6 is known.

$$E_{m_i}^1-E_{m_i}^5 = 0.06 \log K'_{6_i} \tag{VII}$$

K'_{6_i} in Eqn. VII is the apparent equilibrium constant of Reaction 6, where the concentrations of the basic forms of the radical anions are replaced by the total concentrations of the radicals. Some of the reference $E_{m_7}{}^1$ and $E_{m_7}{}^2$ which might be used for evaluation of $E_{m_7}{}^5$ and other systems are summarized in Table I, along with the available data used for this calculation.

RESULTS AND DISCUSSION

None of the primary radicals which are produced by the electron pulse is present in our solutions at the end of the pulse, due to the very fast Reactions 7–10:

$$H_2O \rightsquigarrow e_{aq}^-,\ H,\ OH,\ H_2,\ H_2O_2 \tag{7}$$

$$e_{aq}^{-} \text{ or } H+O_2 \rightarrow O_2^{-} \text{ (or } +H^{+}) \tag{8}$$

$$e_{aq}^{-} \text{ or } H+Q \rightarrow Q^{-} \text{ (or } +H^{+}) \tag{9}$$

$$H \text{ or } OH+HCO_2^{-} \rightarrow H_2 \text{ or } H_2O+CO_2^{-} \tag{10}$$

Furthermore the CO_2^{-} radical produced by Reaction 10 will transfer an electron either to oxygen or to the quinone very rapidly.

$$CO_2^{-}+O_2 \rightarrow O_2^{-}+CO_2 \tag{11}$$

$$CO_2^{-}+Q \rightarrow Q^{-}+CO_2 \tag{12}$$

The only radicals which will be present practically at the end of the pulse under our conditions are therefore O_2^{-} and/or Q^{-}. When *tert*-butanol was present in the solutions all OH radicals will abstract a hydrogen atom from the alcohol yielding the *tert*-butanol radical which is relatively unreactive. We studied the spectra of the semiquinones of 2,5-dimethyl-*p*-benzoquinone (DMBQ) and of 1,4-naphthoquinone-2-sulfonate (NQS) which are shown in Fig. 1. The spectra in deaerated solutions immediately at the end of the pulse closely resembles those obtained 0.75 ms after the end of the pulse in air saturated solutions. We obtain $\varepsilon_{DBMQ^-}^{430} = 7200$ and $\varepsilon_{NQS}^{390} = 10600$ $M^{-1} \cdot cm^{-1}$ similar to the values obtained previously [17]. As noted previously [17] the slow formation of the semiquinone in aerated solutions (cf. insert Fig. 2) must be attributed to the electron transfer from O_2^{-} to the quinone. The following features of the oscillograms similar to the one displayed in Fig. 2 were analyzed: (a) The initial yield of Q^{-} (denoted $A_{t=0}$). (b) The kinetics of electron transfer from O_2^{-} to Q. (c) The final yield of Q^{-} (denoted A_{eq}).

The initial absorption of the semiquinone is attributed to the competition between O_2 and Q for the solvated electrons (and probably a negligible amount of hydrogen atoms). We checked this assumption by studying the dependence of the

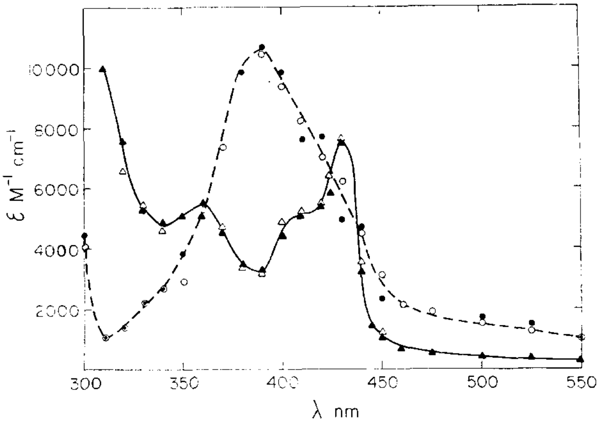

Fig. 1. Spectrum of $DMBQ^{-}$ and NQS^{-} in Ar and in air-saturated solutions. ▲, $2 \cdot 10^{-4}$ M DMBQ, $2 \cdot 10^{-3}$ phosphate buffer (pH 7.2), 0.1 M sodium formate, Ar-saturated; △, $2 \cdot 10^{-4}$ M DMBQ, $2 \cdot 10^{-3}$ phosphate buffer (pH 7.2), 0.1 M sodium formate, air-saturated; ●, $2 \cdot 10^{-4}$ M NQS, $2 \cdot 10^{-3}$ phosphate buffer (pH 7), 1 M *tert*-butanol, Ar-saturated; ○, $2 \cdot 10^{-4}$ M NQS, $2 \cdot 10^{-3}$ phosphate buffer (pH 7), 1 M *tert*-butanol, air-saturated.

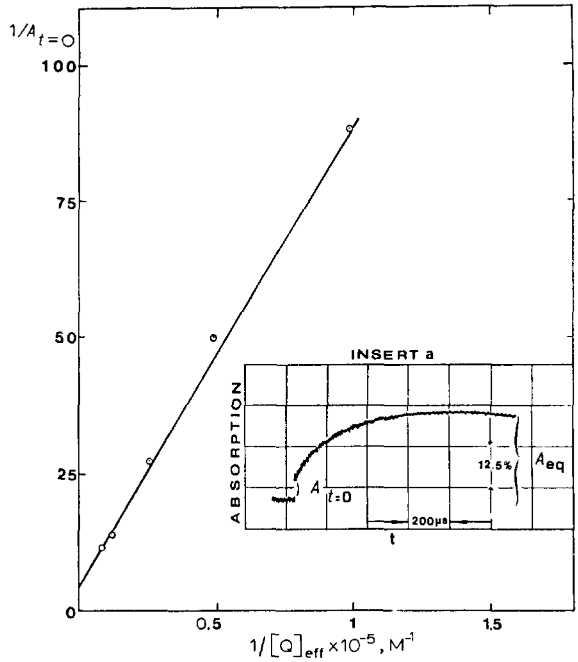

Fig. 2. The dependence of the initial absorbance, $A_{t=0}$ on [DMBQ], air-saturated solutions, with 0.1 M *tert*-butanol and $2 \cdot 10^{-3}$ M phosphate buffer (pH 7), $\lambda = 430$ nm. Insert a: an oscillogram obtained in solution of $2 \cdot 10^{-5}$ M DMBQ, $1.25 \cdot 10^{-4}$ M O_2, 0.1 M sodium formate, $2 \cdot 10^{-3}$ M phosphate buffer (pH 7.2).

initial absorbance on the concentrations of O_2 and Q in solutions of DMBQ containing 0.1 M *tert*-butanol. It was noted that neither the *tert*-butanol radical nor its peroxy radical reacts with benzoquinone [23]. Thus the initial yield should follow Eqn. VIII:

$$\frac{1}{A_{t=0}} = \frac{1}{A^0_{t=0}} + \frac{k_8[O_2]}{A^0_{t=0} k_9[Q]} \qquad \text{(VIII)}$$

where $A_{t=0}$ is the initial absorbance at the end of the pulse and $A^0_{t=0}$ is the absorbance in deaerated solution. Eqn. VIII is shown to be obeyed in Fig. 2. The effective concentration of the quinone, $[Q]_{eff}$, was calculated by subtracting the amount of the semiquinone formed from the initial concentration of the quinone assuming $\varepsilon^{430}_{DMBQ^-} = 7200\ M^{-1} \cdot cm^{-1}$. From the slope of the line in Fig. 2 we obtain $k_8/k_9 = 0.68$ and assuming $k_8 = 1.9 \cdot 10^{10}\ M^{-1} \cdot s^{-1}$ [24], we obtain $k_9 = 2.9 \cdot 10^{10}\ M^{-1} \cdot s^{-1}$. We also measured k_9 directly by following the build up of the absorbance in de-aerated solutions containing variable concentrations of DMBQ and 0.1 M *tert*-butanol. The value thus obtained is $k_9 = 3.1 \cdot 10^{10}\ M^{-1} \cdot s^{-1}$.

The value of K_6 may be determined by studying the dependence of the absorbance of Q^-, at times when the electron transfer reaction 6 is completed (denoted A_{eq}), on the concentration ratio $[O_2]/[Q]$. There are three ways to calculate K_6:

(1) Eqn. IX describes this dependence (provided Equilibrium 6 is achieved which is supported by the forthcoming results):

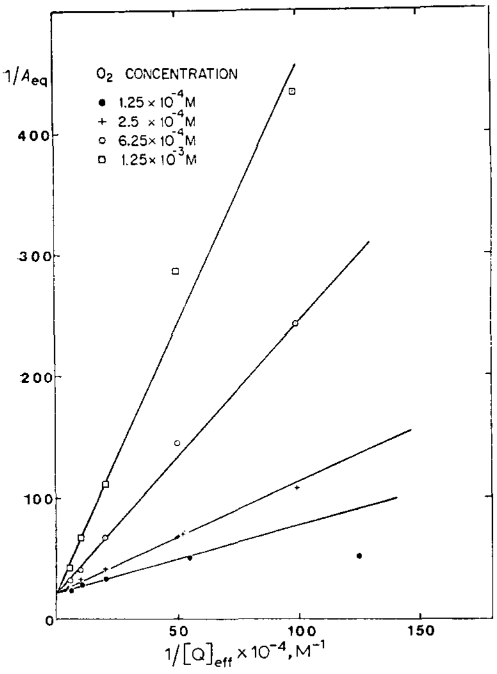

Fig. 3. Determination of the equilibrium constant for the electron transfer between oxygen and DMBQ$^-$ using $1/A_{eq}$ vs. $1/[\mathrm{DMBQ}]$ plots. $2\cdot10^{-3}$ M phosphate buffer (pH 7.2) and 0.1 M sodium formate. ●, $1.25\cdot10^{-4}$ M O_2; +, $2.5\cdot10^{-4}$ M O_2; ○, $6.25\cdot10^{-4}$ M O_2; □, $1.25\cdot10^{-3}$ M O_2.

$$\frac{1}{A_{eq}}=\frac{1}{A^0_{eq}}+\frac{[O_2]}{K_6A^0_{eq}[Q]} \qquad \text{(IX)}$$

where A^0_{eq} is the absorbance of Q$^-$ obtained in the absence of oxygen ($A^0_{eq}=A^0_{t=0}$). This dependence was checked for four different oxygen concentrations and the results obtained are displayed in Fig. 3 to obey Eqn. IX fairly well. All the results from the four different oxygen concentrations give the average value: $K_6=56$.

Using this procedure, the measurements at low concentrations of Q contribute to the line more than do the high concentration measurements: therefore, the results may be sensitive to the correction made for [Q].

(2) We have used the same experimental results to calculate K_6 by a different procedure, this time plotting $[\mathrm{DMBQ}^-]/[O_2^-]$ vs. $[\mathrm{DMBQ}]/[O_2]$ (Fig. 4), assuming again $[Q]=[Q]_0-[\mathrm{DMBQ}^-]$ and $[\mathrm{DMBQ}^-]/[O_2^-]=A_{eq}{}^0/A_{eq}{}^0-A_{eq}$. In this plot, the high concentrations of Q contribute more to the line. The value we get is $K_6=53$.

(3) The kinetics of the relaxation of the above-described system from the initial state to the equilibrium state could be easily followed. Solutions containing the same concentration of oxygen and DMBQ were again employed. The kinetics always followed an observed pseudo-first-order law. The observed rate constant, k_{obs}, should depend on both O_2 and DMBQ concentrations, according to Eqn. X:

$$k_{obs}=k_6[Q]+k_{-6}[O_2] \qquad \text{(X)}$$

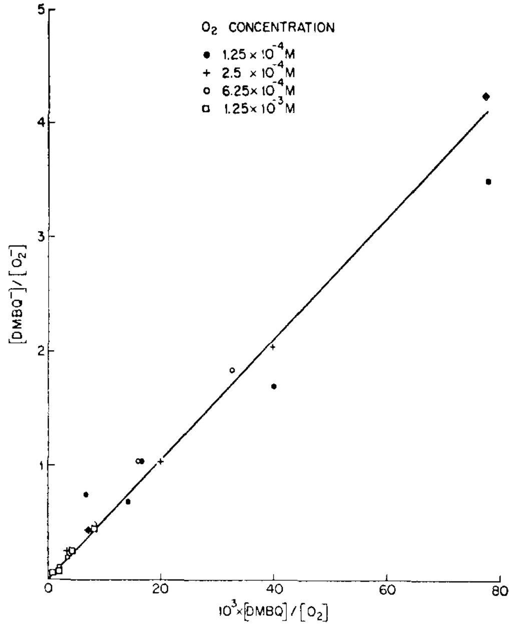

Fig. 4. The dependence of $[\mathrm{DMBQ}^-]/[\mathrm{O}_2{}^-]$ on $[\mathrm{DMBQ}]/[\mathrm{O}_2]$. Symbols and conditions as in Fig. 3.

The results are shown in Fig. 5 where k_{obs} is plotted against the concentration of Q for four different concentrations of oxygen. As expected, four straight parallel lines are obtained, each corresponding to a different concentration of oxygen, with an intercept which equals $k_{-6}[\mathrm{O}_2]$. From the slope of these lines we obtain $k_6 = 1.7 \cdot 10^8$ $\mathrm{M}^{-1} \cdot \mathrm{s}^{-1}$ and $k_{-6} = 4.8 \cdot 10^6$. This yields $K_6 = k_6/k_{-6} = 37$ which is somewhat smaller than the more accurate value obtained by measuring the yields at equilibrium. The value obtained by us for k_6 is nearly half the value obtained by Patel and Willson

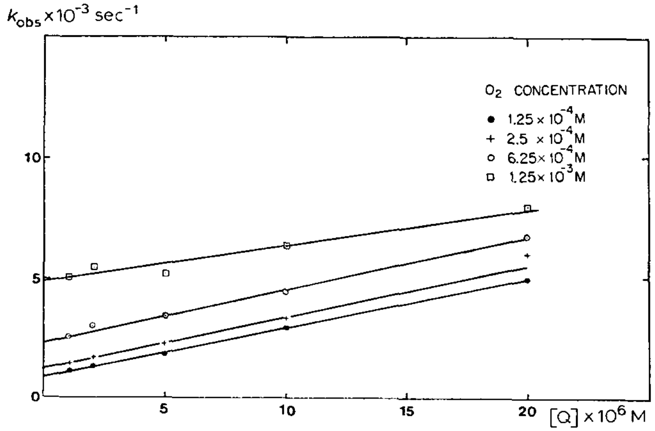

Fig. 5. The dependence of k_{obs} for the electron transfer between O_2 and DMBQ on [DMBQ] and $[\mathrm{O}_2]$. Symbols and conditions as in Fig. 3.

TABLE I

REDOX POTENTIALS OF SOME SEMIQUINONES AND OF THE SUPEROXIDE RADICAL

	pK_{1a}[a]	pK_{2a1}[b]	pK_{2a2}[b]	K_4[b]	E_0^3[c]	E_0^1[d]	E_0^2[d]	$E_{m_7}^3$[c★]	$E_{m_7}^1$[d★]	$E_{m_7}^2$[d★]
p-Benzoquinone	4.1	9.85	11.4	4.2	0.699	0.339	1.041	0.28	0.099	0.459
2-Methylbenzoquinone	4.45	10.05	11.5	2				0.24	0.023	0.46
2,3-Dimethylbenzoquinone	4.65	10.43	12.6	3.6				0.18	−0.074	0.43
2,5-Dimethylbenzoquinone	4.6	10.35	12.5	6	0.590	0.21	0.980	0.176	−0.067	0.42
2,3,5-Trimethylbenzoquinone	4,95	10.8	12.9	3.3				0.11	−0.165	0.385
2,3,5,6-Tetramethylbenzoquinone (duroquinone)	5.1	11.25	13.2	1.3	0.480	0.065	0.895	0.06	−0.24	0.36
2-Methyl-1,4-naphthoquinone (menadione)	4.7							−0.005	−0.20[g]	0.19
1,4-Naphthoquinone-2-sulfonate (NQS)									−0.06[e]	
Vitamin K-1	5.5							−0.06	−0.17[h]	0.22
2,3-Dimethylnaphthoquinone	4.25								−0.24[h]	
O_2 (1 atm)	4.88[f]	11.8[i]							−0.33[e]	

[a] Ref. 17.

[b] Ref. 25.

[c] Ref. 27 or calculated from values cited therein.

[d] Calculated as described in the Procedure section, except when stated otherwise.

[e] Experimentally determined in this study.

[f] Ref. 26.

[g] See details Ref. 18.

[h] K'_6 calculated from ref. 17.

[i] Ref. 43.

★ $E^i_{m_7}$ is referred to as $E^{0\prime}$ in the literature.

[17]. This is at least partially due to negligence of the contribution of the back reaction 6 to the overall rate observed by the latter authors. The best value for K_6 appears to be 50 ± 10, for DMBQ.

The equilibrium constant, K_6, for the electron transfer between O_2^- and NQS was determined as described above. The value obtained is $K_6 = 55$. Fewer experiments were done, therefore we consider this value to be less accurate. Using the value of K_6 for DMBQ we calculate the redox potential of the O_2/O_2^- couple as described in the previous section. Once we have $E_{m_i}{}^1$ for the oxygen system, $E_{m_i}{}^2$ may also be easily calculated, as well as the pH dependence of both potentials. These results are included in Table I. The O_2/O_2^- half-cell reaction may be used as a convenient reference reaction for further calculations of other one-electron redox potentials. This we did for the NQS/NQS^- systems for which we experimentally determined K_6.

The redox potentials of the radicals evaluated

The details of the principles of evaluation of the redox potentials of a radical R have been described in the previous sections. The method is based upon the determination of the equilibrium constant K_{13} of the electron transfer reaction

$$R + A \rightleftarrows R^+ + A^- \qquad (13)$$

Details of the various ways of determining K_{13} were given for the electron transfer from O_2^- to DMBQ. The method is general as long as the following conditions are fulfilled: (a) An acceptor/donor in a suitable E^0 range, is available for which the one-electron redox potential is known. (b) It must be proved that the electron transfer reaction is reversible. This condition was not met with in most of the earlier studies [19–21].

If both these conditions be taken into consideration, K_{13} can be measured and E^0 of the radical can be calculated. An obvious advantage of this method is that no serious errors occur in the determination of E^0 even if an appreciable experimental error should occur in K_{13} itself. An error of 50 % in K_{13} changes E^0 by only 10 mV.

(a) *The redox potentials of several semiquinones.* In this study we have calculated the redox potentials of a few semiquinones. This was done by using both data available in the literature and new measurements.

From previous data [17, 25, 27], we have calculated the redox potentials of a few semiquinones (Table I). Using the calculated $E_{m_7}{}^1$ for duroquinone and K_6 as evaluated by Patel and Wilson [17] we obtained $E_{m_7}{}^5 = -0.31$ V, which is in accordance with our results. Now we could use $E_{m_7}{}^5$ as the reference potential for evaluating other redox potentials. We have calculated K_6 for vitamin K-1 and 2,3-naphthoquinone from the results of Patel and Wilson [17], and calculated accordingly $E_{m_7}{}^1$ for these quinones (Table I). For NQS we have measured experimentally K_6 and calculated $E_{m_7}{}^1$ (Table I).

This method was recently used to determine the redox potentials of a dozen or so nitro compounds [22] and riboflavin.

(b) *The redox potential of O_2.* The values of the redox potentials of the O_2/O_2^- and O_2^-/H_2O_2 couples are of major importance for many inorganic reaction mechanisms and not less for biological mechanisms. The role of O_2^- in many biological processes is well known, and therefore many attempts to calculate or estimate

TABLE II

REDOX POTENTIALS OF THE $O_2/HO_2/H_2O_2$* SYSTEMS

No.	Reaction	E^0 (V)
1	$O_2+e \rightarrow O_2^-$	−0.33
2	$H^+ + O_2 + e \rightarrow HO_2$	−0.037
3	$H^+ + O_2^- + e \rightarrow HO_2^-$	+1.00
4	$H^+ + HO_2 + e \rightarrow H_2O_2$	+1.42
5	$2H^+ + O_2^- + e \rightarrow H_2O_2$	+1.71
6	$H^+ + O_2 + 2e \rightarrow HO_2^-$	+0.34
7	$2H^+ + O_2 + 2e \rightarrow H_2O_2$	+0.69

* $pK_{1a} = 4.88$ [26], $pK_{1a2} = 11.8$ [43].

$E^0(O_2/O_2^-)$ have been made. The values for this potential were widely spread between +0.005 [6] and −0.59 V [9].

Recently Chevalet et al. [12] determined $E^0(O_2/O_2^-)$ by polarography for the first time. They published the value of −0.27 V. (It seems that due to an arithmetic error they published the value of −0.27 V, while their real value is −0.29 V.)

An indirect determination was done by Berdnikov and Zhuravleva [14] from kinetic data on the reaction of Fe^{3+} with H_2O_2 and that of Fe^{2+} with HO_2. They obtained a value of $E^0(O_2/O_2^-) = -0.33$ V. Rao and Hayon [21] published a very different value of +0.15 V. This value was shown [11, 13] to be wrong for various reasons, as discussed in the introduction.

Wood [13] and ourselves [11] recalculated $E^0(O_2/O_2^-)$ from experimental data of Rao and Hayon [21] and of Patel and Wilson [17] and got $E^0(O_2/O_2^-) = -0.33$ V.

TABLE III

REDOX POTENTIAL OF O_2/O_2^-

	$E^0(O_2/O_2^-)$	
$O_2^- + DMBQ^a \rightleftharpoons O_2 + DMBQ^-$	−0.33 V	Determined in this study
Superoxide dismutase Cu(II)+O_2^- Superoxide dismutase Cu(I)	−0.32	Calculated in this study from Ref. 40
$O_2^- + DQ^b \rightleftharpoons O_2 + DQ^-$	−0.31	Ref. 18
$O_2^- + MQ^c \rightleftharpoons O_2 + MQ^-$	−0.325	Ref. 18
$O_2^- + IDS^d \rightleftharpoons O_2 + INS^-$	−0.325	Ref. 18
Polarography studies	-0.29^e (−0.27)	Ref. 12
Cytochrome $c^{2+} + O_2 \rightleftharpoons$ cytochrome $c^{3+} + O_2^-$	−0.27, −0.31	From kinetic studies of forward and backward reactions[f]
Cytochrome $b_5^{2+} + O_2 \rightleftharpoons$ cytochrome $b_5^{3+} + O_2^-$	−0.25, −0.29	

[a] DMBQ, 2.5-dimethylbenzoquinone.

[b] DQ, duroquinone (tetramethylbenzoquinone).

[c] MQ, menadione.

[d] IND, indigodisulfonate.

[e] Chevalet et al. [12] published the value of −0.27 V. This value has an arithmetic error, and their results really yield −0.29 V.

[f] Private communication from I. Yamazaki, submitted to Biochemistry.

We felt it important to redetermine this value experimentally because of its importance and the doubt that in the previous determinations [19–21] equilibrium between O_2^- and the quinone was achieved. In these earlier studies (refs. 19–21 and Rao, P. S. and Hayon, E., personal communication) there was no check of whether equilibrium was achieved. As shown later, in all those studies, except by chance for the O_2^-, equilibrium was not achieved and all redox potentials published are meaningless.

In this study we took great care to prove that equilibrium is reached and thereafter calculated the redox potential of O_2/O_2^- and then that of O_2^-/H_2O_2 as given in Table II.

In Table III the redox potential of O_2/O_2^- from various recent published and unpublished data are summarized.

Redox properties of the superoxide radical in some inorganic systems

The superoxide radical can act as both a reducing (Reaction 1b) and an oxidizing (Reaction 2b) agent:

$$O_2 + e \rightleftarrows O_2^- \tag{1b}$$

$$O_2^- + e \rightleftarrows O_2^{2-} \tag{2b}$$

The oxidation power of the superoxide radical decreases with increasing the pH, i.e. HO_2 is a better oxidant than O_2^- (compare lines 3 and 4 in Table II). On the other hand, O_2^- is a better reducing agent than HO_2 (compare lines 1 and 2 in Table II).

The ability of the superoxide radical to serve as both an oxidizing and a reducing agent is manifested in several inorganic systems, some of which we shall discuss in detail. For example HO_2 can reduce Ce(IV) and oxidize Ce(III) in H_2SO_4, while Tl^{2+} has a peculiar characteristic: it can both oxidize and reduce HO_2, yielding Tl^{3+} and Tl^+, respectively, although thermodynamically the oxidation of Tl^{2+} is more favorable.

We will discuss the properties of O_2^- in two systems in detail: In acid solutions, HO_2 is known to reduce Ce(IV) (refs. 28 and 29 and Faragi, M. and Pecht, I., personal communication). This reaction occurs in $HClO_4$, HNO_3 and H_2SO_4. This reaction is induced by reduction of Ce(IV) with H_2O_2:

$$H_2O_2 + \mathrm{Ce(IV)} \rightleftarrows H^+ + HO_2 + \mathrm{Ce(IV)} \tag{14}$$

$$\mathrm{Ce(IV)} + HO_2 \rightarrow \mathrm{Ce(III)} + H^+ + O_2 \tag{15}$$

The suppression of the rate of the disappearance of Ce(IV) by Ce(III) in H_2SO_4 [29] and HNO_3 (Faragi, M. and Pecht, I., personal communication), was attributed to the back reaction 14 which competes with Reaction 15. However, in $HClO_4$ no such effect of Ce(III) was observed [17]. With the value of $E^0_{HO_2/H_2O_2} = 1.42$ V determined in this study, and the well known redox potentials of $E^0_{\mathrm{Ce(IV)/Ce(III)}} = 1.44$, 1.61 and 1.70 V [10] in H_2SO_4, HNO_3 and $HClO_4$, respectively, this behavior can now be rationalized. The equilibrium constants, K_{14}, calculated from these redox potentials are 2.1, $1.5 \cdot 10^3$ and $4.6 \cdot 10^4$ M^{-1} for the same acids, respectively. Furthermore, from K_{14} and the rate constants of Reaction 14 in those acids ($1 \cdot 10^6$ [29], $4 \cdot 10^5$ (Faragi, M. and Pecht, I., personal communication) and $> 10^8$ [28], respectively) we can calculate the rate constants for the back reaction, k_{-14}, to be $4.8 \cdot 10^5$, $2.7 \cdot 10^2$ and $> 2.2 \cdot 10^{-3}$ $M^{-1} \cdot s^{-1}$ in H_2SO_4, HNO_3 and $HClO_4$, respectively.

Obviously, k_{-14} in $HClO_4$ is low enough to avoid competition between Reactions −14 and 15, but high enough in H_2SO_4. The above-calculated value of k_{-14} in H_2SO_4 is in good agreement with the one we recently measured ($2 \cdot 10^5\ M^{-1} \cdot s^{-1}$ [30]) considering the inaccuracy involved in calculating equilibrium constants from free energies of reactions.

Another case to be discussed is the oxidation-reduction reaction of Fe(III) or of $Fe(CN)_6^{3-}$ by O_2^-. For both cases the reduction of the ferric oxidation state by O_2^- (Reaction 16):

$$Fe(III)+O_2^- \rightleftarrows Fe(II)+O_2 \qquad (16)$$

should be highly favored over the entire pH range. On the other hand, O_2^- (or HO_2) may also oxidize the ferrous oxidation state via Reaction 17.

$$Fe(II)+O_2^-(HO_2) \rightleftarrows Fe(III)+O_2^{2-}(H_2O_2) \qquad (17)$$

However, in alkaline solutions the equilibrium state should favor reduction of Fe(III) by H_2O_2. From the data now available, one can calculate the equilibrium constants as well as the rate constants of both Reactions 16 and 17, in a procedure similar to the one we adopted above or the Ce(IV)/Ce(III) system. These considerations explain the pH dependence of the reduction of ferricyanide by H_2O_2. In neutral solutions, although Equilibrium 17 lies to the right, H_2O_2 does reduce $Fe(CN)_6^{3-}$, since Eqn. 16 is also strongly shifted to the right and O_2^-, which is present at very low concentration, reacts mainly with ferricyanide. The net reaction 18 will thus proceed to the right.

$$2Fe(CN)_6^{3-}+H_2O_2 \rightarrow 2Fe(CN)_6^{4-}+O_2+H^+ \qquad (18)$$

Relevant to this analysis are the case of the catalytic decomposition of H_2O_2 by metal ions such as Cu^{2+}, Fe^{3+}, etc. and the case of the oxidation Fe^{2+} or Cu^+ by oxygen. Using the available redox potentials of the ions concerned and that of O_2, it is now possible to check the role of O_2^- in these systems. For example, the results of Cher and Davidson [31] on the oxidation of Fe^{2+} by O_2 in H_3PO_4 quantitatively agree with the formation of O_2^-, while there may be cases where the kinetic behavior seems to rule out its participation.

The O_2^- in biological systems

The possible role of O_2^- in enzymatic reactions was first proposed by Fridovich and Handler [32, 33]. These authors investigated the reaction of xanthine oxidase on several substrates and demonstrated that O_2^- is generated during the xanthine-xanthine oxidase reaction. Since the role of O_2^- in biological systems was first suggested, many superoxide oxidase enzymatic systems have been isolated and studied: for references on the relevance of O_2^- in biological systems an excellent review by Bors et al. [34] has recently been written.

Several flavin derivatives, after being reduced enzymatically, can reduce O_2 to form O_2^-: for references see ref. 34. This mechanism is similar to that of the electron transfer between semiquinones and O_2 as studied in this paper in non-enzymatic systems.

Another group of enzymes, the superoxide dismutases, was isolated and their catalysis in the recombination of O_2^- were widely studied (references are given in ref. 34). The catalysis mechanism apparently involves the following steps:

$$\mathrm{E-M^{n+}+O_2^-} \longrightarrow \mathrm{E-M^{(n-1)+}+O_2} \tag{19}$$

$$\mathrm{E-M^{(n-1)+}+O_2^-} \xrightarrow{2\mathrm{H}^+} \mathrm{E-M^{M+}+H_2O_2} \tag{20}$$

In enzymatic systems, where O_2^- was observed, it can be reduced to H_2O_2, as with dihydro-orotate oxidase, or it can be oxidized as proposed with catalase or dismutated as with superoxide dismutases.

In the enzymatic activation of O_2 it was generally assumed that it involves the complex formation of O_2 with the metal chelates as, for example, the action of cytochrome *P*-450 [35]. Such complexes may have quite high association constants. (With haemocyanin [36] where $K \approx 10^5\ \mathrm{M}^{-1}$.) Furthermore the formation of an $[\mathrm{M}^{n+1} \cdots \mathrm{O_2}^-]$ complex is generally assumed to be an intermediate step in oxygen activation [35]. Such complexes were also observed with many simple cations such as $\mathrm{Th}^{4+} \cdots \mathrm{HO_2}$, $\mathrm{V}^{5+} \cdots \mathrm{HO_2}$, $\mathrm{U}^{6+} \cdots \mathrm{HO_2}$, $\mathrm{Ti}^{4+} \cdots \mathrm{HO_2}$, $\mathrm{Zr}^{4+} \cdots \mathrm{HO_2}$, $\mathrm{Mo}^{6+} \cdots \mathrm{HO_2}$ and $\mathrm{Ce}^{3+} \cdots \mathrm{HO_2}$ [30, 37–39] and their association constant for the reaction

$$\mathrm{M^{n+}+O_2} \rightleftarrows [\mathrm{M^{n+} \cdots O_2^-}] \tag{21}$$

were as high as 10^5 [30]. The feasibility of the various mechanisms which may evolve O_2^- can be estimated from thermodynamic data, for which $E^0(\mathrm{O_2/O_2^-})$ is essential. Therefore the determination of this redox potential is important. For example, Klug-Roth et al. [40] determined both the rate constant of O_2^- with superoxide dismutase $(\mathrm{E-Cu^{2+}+O_2^-})$ and that of oxygen with the enzyme $(\mathrm{E-Cu^{+}+O_2})$, the respective rate constants are $1.2 \cdot 10^9\ \mathrm{M^{-1} \cdot s^{-1}}$, and $0.44\ \mathrm{M^{-1} \cdot s^{-1}}$. From those values we calculate K_{eq} of the reaction

$$\mathrm{E-Cu^{2+}+O_2} \rightleftarrows \mathrm{E-Cu^{+}+O_2} \tag{22}$$

$K_{\mathrm{eq}} = 2.8 \cdot 10^9$ and ΔE^0 of this reaction is 0.57 V. The redox potential of the enzyme was measured directly at pH 7 to be 0.42 V [41].

From this value and from ΔE^0 we can calculate again $E^0(\mathrm{O_2/O_2^-})$ and get the value of -0.32 V, which is in excellent agreement with our value. This agreement indicates that in this system the electron transfer between O_2^- and the enzyme does not involve a stable complex of $[\mathrm{E-Cu^{2+} \cdots O_2^-}]$ or $[\mathrm{E-Cu^{+} \cdots O_2}]$, otherwise ΔE^0 calculated from the rate constants would not give the correct value for $E^0(\mathrm{O_2/O_2^-})$.

On the other hand the existence of the O_2 or O_2^- complexes in enzymatic reactions is of great interest and should be considered.

Malstrom [42] argues that the reduction of O_2 by cytochrome oxidase is an energetically unfavorable process. Using $E^0_{\mathrm{O_2/O_2^-}} = -0.6$ V and $E^0 = 0.28$ V for the enzyme he concludes that the free energy of the reaction is much too high to allow its occurrence. Even with our value of $E^0_{\mathrm{O_2/O_2^-}} = -0.33$ V ΔG^0 is still high ($\approx$ 14 kcal/mol). However, if the association constant of O_2 or O_2^- with the enzyme is 10^4–10^5 this restriction does not apply any more. Such values for association constants of O_2 or O_2^- are not unreasonable and similar values were determined in related systems [30, 36]. As pointed out by Malstrom [42] this explanation will leave much less free energy for the next reductive steps.

An additional interesting application of this method was recently given concerning the development of improved cell radiosensitizers. Various organic compounds are able to act as hypoic cell radiosensitizers: this has obviously a potential value in radiotherapy. Meisel and Neta [44] and Wardman et al. [45] found strong correlations by measuring the one-electron redox potentials of various radiosensitizers by our method, and by comparing it with the radiosensitizing efficiencies. This approach is promising to look for more effective radiosensitizers according to the one-electron redox potentials.

Applications of pulse radiolysis to studies of biological interest

The pulse radiolysis method has many potential uses in biology. This method is capable of producing very unstable and reactive intermediates at reasonable concentrations (10^{-5}–10^{-6} M) within a very short time. Previously the method was mainly used for the study of mechanisms of radicals in inorganic and simple organic systems. Recently, more and more applications to biology have been found and the emphasis has changed to exploiting this tool in biological areas.

In this study it is demonstrated how redox potentials of reactive short living intermediates, of biological interest can be determined. The method is capable, of course, of studying other properties of such intermediates, such as spectra and pK values of the intermediates as well as kinetics of this intermediate with various substrates.

For example the study of the kinetics of peroxide dismutase with O_2^- [40] is possible with this method, where other methods are incapable of following this process.

Many fast reactions of biological interest are studied with flow methods. In the flow method, the substrate with an oxidative of reductive reagent are mixed and the rate of the reaction is followed, provided the reaction is not too fast. ($t_{\frac{1}{2}}$ should be longer than 10^{-3} s) The pulse radiolysis method is faster than the flow methods by more than three orders of magnitude, therefore the range of this method exceeds that of flow methods.

The reactive oxidant or reductant can be formed in situ in the presence of substrate in less than 10^{-6} s. This is done by producing the reductant or oxidant through reducing by e_{aq}^- or oxidizing by OH radicals the stable oxidized or reduced form of the reactant in the presence of the substrate. An example of this application is the reaction of oxygen with myoglobin. This reaction can be easily studied when a mixture of oxygen with metmyoglobin is pulse radiolyzed. The immediate result of the pulse, under appropriate conditions, will cause the reduction of metmyoglobin into myoglobin, in the presence of oxygen. After the formation of myoglobin, one can follow its reaction with O_2 [46].

ACKNOWLEDGEMENT

Supported in part by the U.S. Atomic Commission, contract E (11-1)-3221.

REFERENCES

1 Michaelis, L. (1932) J. Biol. Chem. 96, 703–715
2 Michaelis, L. (1935) Chem. Rev. 16, 243–286
3 Elema, B. (1933) J. Biol. Chem. 100, 149–153
4 Czapski, G. (1971) Annu. Rev. Phys. Chem. 171–208
5 Klug, D., Rabani, J. and Fridovich, I. (1972) J. Biol. Chem. 247, 4839–4842
6 Jacq, J. and Bloch, P. (1970) Electrochim. Acta 15, 1945–1966
7 Bennet, L. E. (1973) Prog. Inorg. Chem. 18, 1–176
8 Baxendale, J. H., Ward, M. D. and Wardman, P. (1971) Trans. Faraday Soc. 67, 2532–2537
9 George, P. (1965) in Oxidases and Related Redox Systems (King, T. E., Mason, H. S. and Morrison, M., eds.), pp. 3–36, Wiley and Sons, New York
10 Latimer, W. M. (1952) The Oxidation States of Elements and Their Potentials in Aqueous Solution, Prentice Hall, London
11 Ilan, Y. A., Meisel, D. and Czapski, G. (1974) Isr. J. Chem. 12, 891–895
12 Chevalet, J., Ronelle, F., Gierst, L. and Lambert, J. P. (1972) J. Electroanal. Chem. 39, 201–216
13 Wood, P. M. (1974) FEBS Lett. 44, 22–24
14 Berdinkov, V. M. and Zhuravleva, O. S. (1972) Russ. J. Phys. Chem. 46, 1521–1523
15 Morton, R. A. (1965) Biochemistry of Quinones, Academic Press, London
16 Patai, S. (1974) The Chemistry of the Quinoid Compound, John Wiley and Sons
17 Patel, K. B. and Wilson, R. L. (1973) J. Chem. Soc. Faraday Trans. I, 69, 814–825
18 Meisel, D. and Czapski, G. (1975) J. Phys. Chem. 79, 1503–1509
19 Rao, P. S. and Hayon, E. (1974) J. Am. Chem. Soc. 96, 1287–1294
20 Rao, P. S. and Hayon, E. (1974) J. Am. Chem. Soc. 96, 1295–1300
21 Rao, P. S. and Hayon, E. (1973) Biochem. Biophys. Res. Commun. 51, 468–473
22 Meisel, D. and Neta, P. (1975) J. Am. Chem. Soc. 79, 5198–5203
23 Simic, M. and Hayon, E. (1973) Biochem. Biophys. Res. Commun. 50, 364–368
24 Anbar, M., Bambenek, M. and Ross, A. B. (1974) Selected Specific Rates of Reactions, NBS Report No. NSROS-NBS43
25 Bishop, C. A. and Tong, L. K. J. (1965) J. Am. Chem. Soc. 87, 501–505
26 Behar, D., Czapski, G., Rabani, J., Dorfman, L. M. and Schwarz, H. A. (1970) J. Phys. Chem. 74, 3209–3213
27 Clark, W. M. (1960) Oxidation Reduction Potentials of Organic Systems, The Williams & Wilkins Corp.
28 Samuni, A. and Czapski, G. (1970) Isr. J. Chem. 8, 551–561
29 Czapski, G., Bielski, B. H. and Sutin, N. (1963) J. Phys. Chem. 67, 201–203
30 Meisel, D., Ilan, Y. A. and Czapski, G. (1974) J. Phys. Chem. 78, 2330–2334
31 Cher, M. and Davidson, N. (1955) J. Am. Chem. Soc. 77, 793–798
32 Fridovich, I. and Handler, P. (1958) J. Biol. Chem. 233, 1578–1580
33 Fridovich, I. and Handler, P. (1958) J. Biol. Chem. 233, 1581–1585
34 Bors, W., Saran, M., Lengfeldon, E., Spött, R. and Michel, C. (1974) Curr. Topics Rad. Res. Q. 9, 247–309
35 Ullvich, V., Ruf, H. H. and Mimonn, H. (1972) in Biological Hydroxylation Mechanisms (Boyd, G. S. and Smellie, R. M. S., eds.), pp. 11–19, Academic Press
36 Erel, Z., Shaklai, N. and Daniel, E. (1972) J. Mol. Biol. 64, 341–352
37 Samuni, A. and Czapski, G. (1970) J. Phys. Chem. 74, 4592–4595
38 Meisel, D., Levanon, H. and Czapski, G. (1974) J. Phys. Chem. 78, 779–782
39 Meisel, D., Czapski, G. and Samuni, A. (1973) J. Am. Chem. Soc. 95, 2786–2790
40 Klug-Roth, D., Fridovich, J. and Rabani, J. (1973) J. Am. Chem. Soc. 95, 2786–2790
41 Fee, J. A. and Dicorleto, P. E. (1973) Biochemistry 12, 4893–4899
42 Malstrom, G. B. (1974) Q. Rev. Biophys. 6, 389–431
43 Shumb, W. C., Satterfield, C. N. and Wentworth, R. L. (1955) in Hydrogen Peroxide, Reinhold Publishing Corporation, New York
44 Meisel, D. and Neta, P. (1975) E. J. Hart Int. Symp. Rad. Chem., Argonne, Ill., U.S.A.
45 Wardman, P., Adams, G. E., Fluckart, I. R., Stratford, I. J. and Watts, M. E. (1975) E. J. Hart Int. Symp. Rad. Chem., Argonne, Ill., U.S.A.
46 Ilan, Y. A. and Czapski, G. (1975) Biochim. Biophys. Acta, submitted for publication