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## Redox Potentials of Free Radicals. IV. Superoxide and Hydroperoxy Radicals $\cdot O_2^-$ and $\cdot HO_2$

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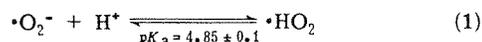
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The fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry was used to generate  $\cdot O_2^-$  and  $\cdot HO_2$  radicals in aqueous solution, and follow their one-electron redox reactions. In the presence of electron acceptors A, the one-electron oxidation of these radicals was monitored by following the formation of  $\cdot A^-$  radicals:  $\cdot O_2^- + A \rightleftharpoons O_2 + \cdot A^-$ . Similarly, the one-electron reduction of  $\cdot O_2^-$  was monitored in the presence of electron donors:  $\cdot O_2^- + AH_2 \rightarrow H_2O_2 + \cdot A^-$ . The efficiencies of these electron transfer processes were found to be dependent on the two-electron redox potentials of the electron donor or acceptor compounds used. From the midpoint (50% electron transfer) of these titration curves the kinetic potentials of some redox couples could be derived (at pH 7.0,  $\sim 25^\circ$ ):  $E_k^{01} = +0.07$  V for the  $O_2/\cdot O_2^-$  couple and  $E_k^{01} \geq +0.36$  V for the  $\cdot O_2^-/H_2O_2$  couple. The oxidation of  $\cdot O_2^-$  was found to take place under reversible equilibrium conditions, whereas the reduction of  $\cdot O_2^-$  was not reversible under the experimental conditions used. The observed rate constants of electron transfer reactions involving  $\cdot O_2^-$ ,  $\cdot HO_2$ , and  $O_2$  with a large number of acceptors and donors have been tabulated and found to be in general agreement with the  $E_k^{01}$  values derived for the appropriate redox couples.

### Introduction

Many chemical and biological oxidation-reduction reactions occur *via* the intermediary of molecular oxygen, which can act as a mediator in electron transfer processes.<sup>2-4</sup> Many reactions involving oxygen in the ground state also proceed by one-electron steps *via* the superoxide radical  $\cdot O_2^-$ . The role and importance of  $\cdot O_2^-$  in certain enzymatic and nonenzymatic oxidation reactions have been established (see, *e.g.*, ref 3, 5, and 6). The mechanism of re-oxidation by oxygen of reduced biochemical systems (*e.g.*, reduced flavins,  $Fe^{2+}$  cytochromes, NADH, leuco dyes) has been shown or suggested to proceed in some cases *via* the formation of  $\cdot O_2^-$  radicals.

The superoxide radical can undergo acid-base reactions, reaction 1,<sup>7,8</sup> and the physicochemical properties of  $\cdot O_2^-$



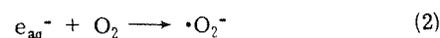
and  $\cdot HO_2$  radicals have recently been reviewed.<sup>9,10</sup> The impression has existed among some physical chemists that these radical intermediates are relatively unreactive species. Biochemical studies,<sup>3,5,6</sup> however, have shown that the one-electron oxidation *and* reduction reactions of  $\cdot O_2^-$

and  $\cdot HO_2$  can be of vital importance in certain biological reactions. No direct experimental determination of the redox potentials of the  $\cdot O_2^-/O_2$ ,  $\cdot O_2^-/H_2O_2$ ,  $\cdot HO_2/O_2$ , and  $\cdot HO_2/H_2O_2$  couples have been obtained, leading to considerable uncertainties in establishing the reaction mechanism of chemical and biological oxidation processes. This work is an attempt to determine experimentally the redox potentials of these couples, based on a method recently developed in this laboratory.<sup>11,12</sup> Preliminary results have been published.<sup>13</sup>

### Experimental Section

The  $\cdot O_2^-$  and  $\cdot HO_2$  radicals were produced in aqueous solution using the technique of pulse radiolysis. The details of the experimental set-up used have been described.<sup>14,15</sup>

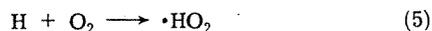
The superoxide radical was produced from the reaction of oxygen with hydrated electrons



under conditions such that all (>90%) the  $e_{aq}^-$  reacted with  $O_2$  and none with the electron acceptor (A) or electron donor ( $AH_2$ ) molecules used

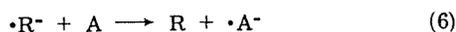


The concentrations of  $O_2$ ,  $A$ , and  $AH_2$  were adjusted based on the known<sup>16</sup> rate constants  $k_2$ ,  $k_3$ , and  $k_4$ . The OH radicals produced from the radiolysis of water were, in some cases, scavenged<sup>14</sup> by *tert*-butyl alcohol. The radical produced from this alcohol can react with oxygen to form the corresponding peroxy radical.<sup>14</sup> This peroxy radical does not undergo<sup>14,17,18</sup> acid-base reactions up to  $pH \geq 13$ , and was found not to interfere with the results obtained in this work (see ref 13 and below). The H atoms produced from the radiolysis of water react mainly with  $O_2$



Depending on the pH of the experiments, the radicals were present either as  $\cdot O_2^-$  or  $\cdot HO_2$ , based on equilibrium 1.

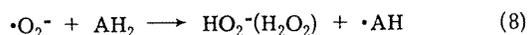
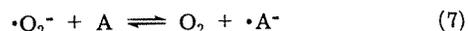
The details of the method used to determine the potentials of organic free radicals ( $\cdot R^-$ ) have been described.<sup>11</sup> This method depends on following the formation of the radicals produced by electron transfer to the acceptors (A), or by electron transfer from the donors ( $AH_2$ ), *e.g.*



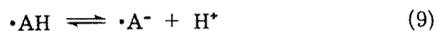
From a plot of efficiency of formation of  $\cdot A$  radicals (expressed as percentage) as a function of the known redox potentials of the acceptors, typical titration curves are obtained. From the midpoint (50% electron transfer) of these curves, the potentials of the  $\cdot R^-$  radicals can be derived. The redox potentials of the acceptors determined experimentally<sup>19</sup> are usually for two-electron processes.

It has subsequently been shown<sup>11b</sup> that for most of the organic free radicals examined equilibrium conditions did not prevail for reaction 6, under the experimental conditions used. Hence, the Nernst equation cannot be applied and the potentials derived from this method have been referred to as *kinetic potentials*,<sup>11b</sup> since it characterizes the efficiency of a radical to reduce an acceptor (or oxidize a donor) in kinetic competition with other reactions.<sup>11b</sup>

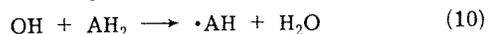
For the  $\cdot O_2^-$  radical, it will be shown below that reversible conditions do appear to exist for the one-electron oxidation of  $\cdot O_2^-$ , equilibrium 7, but not for the one-electron



reduction of  $\cdot O_2^-$ , reaction 8. The  $\cdot AH$  radical produced can also undergo acid-base equilibration.



The percentage efficiencies of the electron transfer processes were obtained based on determination of the 100% efficiency ("blank") for formation of the  $\cdot A^-$  or  $\cdot AH$  radicals, according to reactions 3 and 10. These "blanks" were



carried out for every acceptor and donor compound each time just prior to carrying out the experiment, under identical experimental conditions of concentration, dose, pH, wavelength, etc. The lowest possible concentration of radicals were used to minimize radical-radical reactions.

Table I lists the acceptors and donors used, and the  $E^{01}$  values taken from the literature.<sup>19</sup> The notation  $E^{01}$  is the value at  $pH 7.0$  and  $\sim 25^\circ$ , and  $E_m$  the value at the stated pH of the experiment. The sign of the potentials is in accord with the IUPAC convention.

TABLE I: List of Electron Donors and Acceptors Used

Compound	$E^{01},^a$ V	$\lambda,^b$ nm monitored
1. <i>N</i> -Ethylmaleimide	-0.500	335
2. Carbon dioxide	-0.420	
3. NAD <sup>+</sup>	-0.320	400
4. Lipoate	-0.289	385
5. Anthraquinone-2,6-disulfonate	-0.184	400
6. 2,3-Dimethylnaphthaquinone	-0.070	
7. Vitamin K	-0.060	
8. Cytochrome $b_5$	-0.055	
9. Menquinone	+0.002	400
10. Duro- <i>p</i> -hydroquinone <sup>c</sup>	+0.042	
11. Methylene Blue	+0.011	580
12. Toluidine Blue O	+0.034	610
13. Duroquinone	+0.068	445
14. Indophenol (pH 9)	+0.089	610
15. Trimethyl- <i>p</i> -hydroquinone	+0.108	430
16. 1,4-Naphthaquinone-2-sulfonate	+0.118	400
17. 1,2-Naphthaquinone	+0.143	290
18. 2,5-Dimethyl- <i>p</i> -hydroquinone <sup>c</sup>	+0.170	
19. 1,2,4-Trihydroxybenzene <sup>c</sup>	+0.176	
20. 2,5-Dimethyl- <i>p</i> -benzoquinone	+0.176	440
21. 1,2-Naphthaquinone-4-sulfonate	+0.217	290
22. Methyl- <i>p</i> -hydroquinone <sup>c</sup>	+0.233	430
23. 2-Methyl- <i>p</i> -benzoquinone	+0.240	
24. <i>p</i> -Hydroquinone <sup>c</sup>	+0.280	430
25. 2,5-Dichloro- <i>p</i> -hydroquinone <sup>c</sup>	+0.285	
26. <i>p</i> -Benzoquinone	+0.293	430
27. 2,5-Dichloro- <i>p</i> -benzoquinone	+0.310	430
28. <i>N</i> -Methyl- <i>p</i> -phenylenediamine	+0.330	520
29. 3-Dimethyl- <i>p</i> -aminophenol	+0.410	385
30. 2,3-Dicyano- <i>p</i> -hydroquinone <sup>c</sup>	+0.510	430
31. Diphenquinone	+0.534	400
32. <i>p</i> -Methoxyphenol	+0.564	400

<sup>a</sup> Redox potential at  $pH 7.0$  and  $25^\circ$ ; values derived from ref 19.

<sup>b</sup> See also ref 11. <sup>c</sup> Compounds used in Figure 2.

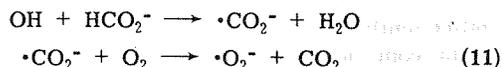
The chemicals used were the highest research grade commercially available, and were obtained from Aldrich, Eastman Chemicals, J. T. Baker, K and K Laboratories, and Baker and Adamson. The solutions were buffered with  $\sim 1$  mM phosphate, and perchloric acid and potassium hydroxide were used to adjust the pH. Special care was taken to reduce exposure of the solutions to light.

## Results

*Determination of the Potential of the  $\cdot O_2^-|H_2O_2$  Couple.* The one-electron reduction of the  $\cdot O_2^-$  radical gives  $O_2^{2-}$  which is rapidly protonated by water to give hydrogen peroxide ( $pK_a^1$  of  $H_2O_2 = 11.75$ ). The electron donors chosen to bring about this reduction, reaction 8, were various hydroquinones, phenols, and *N*-methyl-*p*-phenylenediamine. These were selected on the basis of (a) their stability in oxygenated solutions, and (b) the formation of radical intermediates which absorb at appropriate wavelengths, react very slowly with  $O_2$ , and have relatively high molar extinction coefficients. The intermediates produced from these donors are semiquinone radicals (from hydroquinones), phenoxy radicals (from phenols), and aromatic amine cation radicals (from phenylenediamines).

These experiments were carried out in buffered solution at  $pH 7.0$ , oxygen-saturated ( $[O_2] = 1.25 \times 10^{-3} M$ ) and

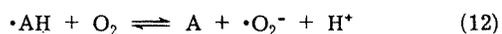
typically 1.0 mM concentration of the electron donors. The OH radicals produced from the radiolysis of water were "converted" to  $O_2^-$  radicals by carrying out the experiments in the presence of 0.1 M sodium formate, when the following reactions occur



Under the experimental conditions chosen, none of the OH radicals react with  $\text{AH}_2$  molecules and all the  $\cdot\text{CO}_2^-$  radicals form  $\cdot\text{O}_2^-$  in  $\ll 1 \mu\text{sec}$ , since  $k_{11}$  was found to be  $\sim 5.0 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ .

The percentage efficiencies for electron transfer from the donor molecules to the superoxide radical  $\cdot\text{O}_2^-$  are presented, in Figure 1a, as a function of the redox potential of the donor compounds. It is seen that with increase (more positive) in the  $E^{01}$  of the  $\text{AH}_2$  molecules the percentage efficiency decreases from  $\sim 100$  to 0%. A "titration" type of curve is obtained. It was, unfortunately, not possible to find suitable donors with  $E^{01}$  values in the range  $+0.3$  to  $+0.4$  V.

It should be pointed out that, under the experimental conditions used, the  $\cdot\text{AH}$  or  $\cdot\text{A}^-$  radicals produced in reaction 8, namely, semiquinones, phenoxy, and aromatic amine cations radicals, react relatively slowly with  $\text{O}_2$ . Therefore, at the time scale of the experimental measurement essentially none had reacted *via*

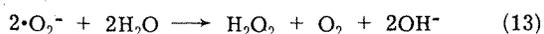


Indeed, as will be shown below, the back reaction 12 is prevalent.

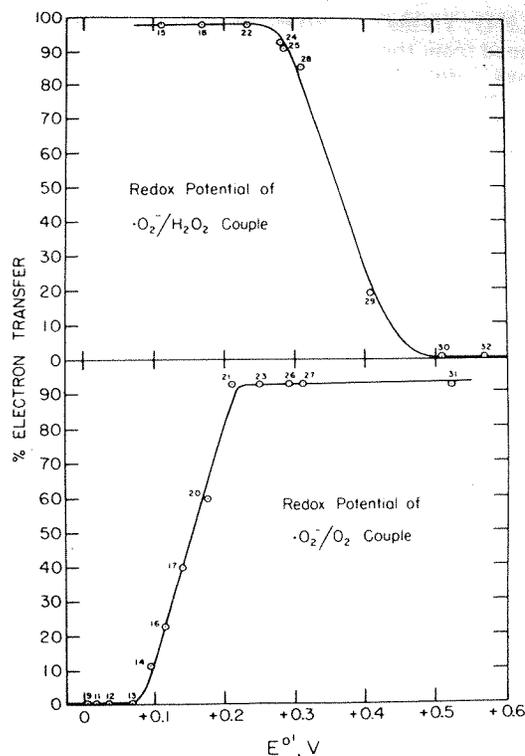
The rate constant of the reaction of  $\cdot\text{AH}$  or  $\cdot\text{A}^-$  radicals with  $\text{H}_2\text{O}_2$ , the reverse reaction 8, is considerably slower than the forward reaction. Hence reversible equilibrium conditions do not exist for the one-electron reduction of  $\cdot\text{O}_2^-$  by  $\text{AH}_2$ , and the Nernst equation cannot be applied. Similar conditions were found<sup>11,12</sup> for the one-electron oxidation of organic free radicals, and the potentials of such couples have been referred to as kinetic potentials.<sup>11b</sup>

From Figure 1a, the midpoint (*i.e.*, 50% electron transfer) on the titration curve corresponds to a  $E_k^{01}$  value of  $\geq +0.36$  V, for the  $\cdot\text{O}_2^-/\text{H}_2\text{O}_2$  redox couple. This value is based on the two-electron redox potential of the  $\text{AH}_2$  donors. It is considered to be a lower limit since insufficient data were available for its determination. The  $E^{01}$  value (*i.e.*, the thermodynamic redox potential) for this couple is expected to be higher (*i.e.*, more positive), see more below.

**Determination of the Potential of the  $\text{O}_2^-/\cdot\text{O}_2^-$  Couple.** The one-electron oxidation of the  $\cdot\text{O}_2^-$  radical, reaction 7, can be studied by following the formation of the  $\cdot\text{A}^-$  radicals from various electron acceptors A. These experiments were carried out in oxygen-saturated solutions at pH 7.0 in the presence of 1.0 M *tert*-butyl alcohol to scavenge the OH radicals. A few experiments were carried out using 0.1 M  $\text{HCO}_2^-$  as an OH radical scavenger, or a mixture of 13 mM  $\text{N}_2\text{O}$  and 0.7 mM  $\text{O}_2$ . Essentially similar results were obtained. The concentration of the acceptors used was  $5 \times 10^{-5}$  M (except  $2.0$ – $2.5 \times 10^{-5}$  M when dyes were used), and relatively low  $[\cdot\text{O}_2^-]$  of  $\sim 1$ – $2 \mu\text{M}$ . Under these conditions, essentially none of the  $\cdot\text{O}_2^-$  radicals decayed before reaction with the acceptors since reaction 13 is relatively very slow<sup>7</sup>



The dependence of the efficiency of reaction 7 upon the redox potentials of the acceptors used is given in Figure 1b.



**Figure 1.** (a) The efficiency (expressed as percentage) of electron transfer from donor molecules to  $\cdot\text{O}_2^-$  radicals, and (b) the efficiency of electron transfer from  $\cdot\text{O}_2^-$  to a range of acceptor molecules. Experiments were carried out at pH 7.0 and relatively low concentrations of radicals. For the reduction of  $\cdot\text{O}_2^-$ ,  $[\text{radical}] = 8$ – $12 \mu\text{M}$ , while for the oxidation of  $\cdot\text{O}_2^-$ ,  $[\text{radical}] = 1$ – $2 \mu\text{M}$ . The donors and acceptors used are listed in Table I.

In this case a complete range of acceptors was available. Furthermore, reversible equilibrium conditions were found to exist, under our experimental conditions, for reaction 7.

The two half-cell reactions for this couple are



and

$$E_A^0 - E_{\text{O}_2}^0 = 0.059 \log \frac{[\cdot\text{A}^-][\text{O}_2]}{[\cdot\text{O}_2^-][\text{A}]} \quad (\text{A})$$

Under equilibrium conditions at the midpoint (50% electron transfer) of the titration curve, eq B can be derived

$$E_{\text{O}_2}^0 = E_A^0 - 0.059 \log \frac{2[\text{O}_2]_0 - [\cdot\text{O}_2^-]_0}{2[\text{A}]_0 - [\cdot\text{O}_2^-]_0} \quad (\text{B})$$

where the subscripts "0" denote initial concentrations. Under the experimental conditions used,  $[\text{A}]_0/[\cdot\text{O}_2^-]_0 \sim 12$ – $15$  and eq B can be reduced to

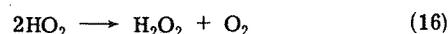
$$E_{\text{O}_2}^0 = E_A^0 - 0.082 \quad (\text{C})$$

From Figure 1b, a midpoint potential value of  $+0.15 \pm 0.02$  V can be obtained, giving a  $E_k^{01} = +0.07 \pm 0.02$  V for the  $\text{O}_2^-/\cdot\text{O}_2^-$  redox couple. This value is based on the two-electron redox potential of the electron acceptors used. The earlier value given<sup>13</sup> for this couple was not corrected on the basis of eq C.

**Determination of the Potential of the  $\cdot\text{HO}_2/\text{H}_2\text{O}_2$  Couple.** The hydroperoxy radicals decay bimolecularly with  $k_{16} = 2.0 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$ , which is considerably faster

**TABLE II: Yields (*G* Values) of Hydrogen Peroxide Produced from the  $^{60}\text{Co}$   $\gamma$  Radiolysis of Oxygenated Aqueous Solutions of Various Hydroquinones at pH  $\sim 0.4$**

Donor, AH <sub>2</sub>	$E_2^{01}$ , V	$G(\text{H}_2\text{O}_2)$	Ref
Duro- <i>p</i> -hydroquinone	+0.042	$\sim 4.40$	This work
2,3-Dimethyl- <i>p</i> -hydroquinone	+0.170	4.44	20
1,2,3-Trihydroxybenzene	+0.176	4.17	20
Methyl- <i>p</i> -hydroquinone	+0.233	$4.4 \pm 0.5$	This work
<i>p</i> -Hydroquinone	+0.280	$4.0 \pm 0.5$	21
2,5-Dichloro- <i>p</i> -hydroquinone	+0.285	2.38	20
		$3.2 \pm 0.4$	22
		$2.30 \pm 0.3$	This work
2,3-Dicyano- <i>p</i> -hydroquinone	+0.510	$2.2 \pm 0.3$	This work



than the decay of the conjugate base  $\cdot\text{O}_2^-$ . The rate constants for the one-electron reduction of  $\cdot\text{HO}_2$  by various donors AH<sub>2</sub>, reaction 17, are relatively low (see below).



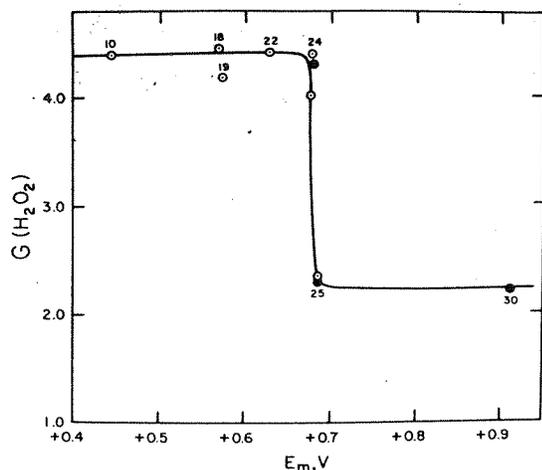
Consequently, using this fast-reaction technique experimental conditions could not be adjusted to determine the potential of the  $\cdot\text{HO}_2/\text{H}_2\text{O}_2$  couple, since reaction 17 cannot compete with (16) at the relatively high concentrations of  $\cdot\text{HO}_2$  ( $\sim 8\text{--}12 \mu\text{M}$ ) necessary for this work.

A different approach was used instead: at relatively very low steady-state concentrations of  $\cdot\text{HO}_2$  radicals, as in  $^{60}\text{Co}$   $\gamma$  radiolysis, the yield of  $\text{H}_2\text{O}_2$  should double whenever reaction 17 takes place with 100% efficiency. Table II gives the yields (*G* values) of hydrogen peroxide determined in oxygenated acid solutions of various hydroquinones and phenols. Included are results from the literature.<sup>20-22</sup>

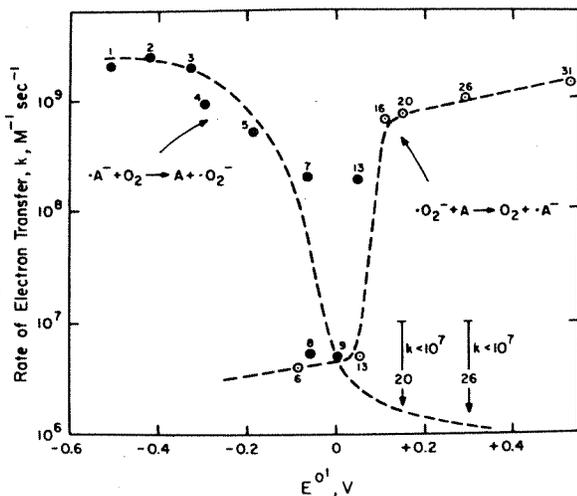
**TABLE III: Rate Constants of Electron Transfer Reactions Involving  $\cdot\text{O}_2^-$ ,  $\cdot\text{HO}_2$ , and  $\text{O}_2$  in Aqueous Solutions**

System <sup>a</sup>	$E^{01}$ , V <sup>b</sup>	$k_{\text{obsd}}$ (electron transfer), $\text{M}^{-1} \text{sec}^{-1}$	Ref
Reaction $\cdot\text{O}_2^- + \text{A} \rightleftharpoons \text{O}_2 + \cdot\text{A}^-$			
NADH	-0.325	$\ll 27$	25
Menaquinone	+0.002	Very low	26
Duroquinone	+0.068	$5.0 \times 10^6$	26
1,4-Naphthaquinone-2-sulfonate	+0.118	$6.6 \times 10^8$	13
2,5-Dimethylbenzoquinone	+0.176	$3.6 \times 10^8$ ; $7.5 \times 10^8$	26,13
Cytochrome C	+0.270	$1.1 \times 10^9$	34
<i>p</i> -Benzoquinone	+0.293	$1.0 \times 10^9$	26,13
Tetranitromethane	+0.310	$2.0 \times 10^9$	27
Diphenoquinone	+0.534	$1.4 \times 10^9$	13
Br <sub>2</sub>	+0.650	$5.6 \times 10^9$	24
Reaction $\cdot\text{O}_2^- + \text{AH}_2 \rightarrow \text{HO}_2^- + \cdot\text{AH}$			
Methyl- <i>p</i> -hydroquinone	+0.233	$1.7 \times 10^7$	<i>c</i>
<i>p</i> -Hydroquinone	+0.280	$1.7 \times 10^7$	<i>c</i>
2,5-Dichloro- <i>p</i> -hydroquinone	+0.285	$1.3 \times 10^7$	<i>c</i>
Reaction $\text{A}^- + \text{O}_2 \rightleftharpoons \text{A} + \cdot\text{O}_2^-$			
(Acetophenone) <sup>-</sup>	-1.29	$2.3 \times 10^9$	28
(Thymine) <sup>-</sup>		$3.5 \times 10^9$	28
(Adenine) <sup>-</sup>		$3.5 \times 10^9$	28
( <i>N</i> -Methylmaleimide) <sup>-</sup>		$2.0 \times 10^9$	29
NAD <sup>•</sup>	-0.325	$1.9 \times 10^9$	30
(Lipoate) <sup>-</sup>		$9.0 \times 10^8$	31
(9,10-Anthraquinone-2-sulfonate) <sup>-</sup>	-0.225	$4.6 \times 10^8$	32
(9,10-Anthraquinone-1-sulfonate) <sup>-</sup>	-0.218	$4.2 \times 10^8$	32
(9,10-Anthraquinone-2,6-disulfonate) <sup>-</sup>	-0.184	$5.0 \times 10^8$	33
(Vitamin K) <sup>-</sup>	-0.06	$2.0 \times 10^8$	26
Cytochrome b <sub>5</sub>	$\sim -0.06$	$\sim 5.0 \times 10^6$	34
(Menaquinone) <sup>-</sup>	+0.002	$\sim 5.0 \times 10^6$ <sup>e</sup>	34
(Duroquinone) <sup>-</sup>	+0.05	$2.0 \times 10^8$	26
(2,5-Dimethyl- <i>p</i> -benzoquinone) <sup>-</sup>	+0.176	$\ll 1.0 \times 10^7$	26
( <i>p</i> -Benzoquinone) <sup>-</sup>	+0.293	$\ll 1.0 \times 10^7$	26
Reaction <sup>d</sup> $\cdot\text{HO}_2 + \text{A} \rightleftharpoons \text{O}_2 + \cdot\text{AH}$			
Cytochrome C	+0.254	Unreactive	35
Tetranitromethane	+0.310	$5.6 \times 10^5$	27
Br <sub>2</sub>	+0.650	$1.1 \times 10^8$	24

<sup>a</sup> Solution at near neutral pH, unless stated otherwise. <sup>b</sup> Redox potential of parent compounds at pH 7.0, 25°, from ref 19. <sup>c</sup> This work. <sup>d</sup> Acidic Solutions, pH 0.4 to 2.0. <sup>e</sup> This value is surprisingly low.



**Figure 2.** Dependence of  $G(\text{H}_2\text{O}_2)$ , produced from the  $^{60}\text{Co}$   $\gamma$  radiolysis of air-saturated aqueous solutions of various hydroquinones and phenols at pH  $\sim 0.4$ , upon the redox potential of the acceptors used. Open symbols are from ref 20–22, and closed symbols from this work.



**Figure 3.** Dependence of the reaction rate constants of electron transfer processes involving  $\text{O}_2$  and  $\cdot\text{O}_2^-$  upon the redox potential of the acceptor or donor molecules. See Table III for details and references to literature values.

The  $G(\text{H}_2\text{O}_2)$  yields were found to decrease with increase in the redox potential of the  $\text{AH}_2$  donors. These results are presented in Figure 2. An abrupt change in the yields of  $\text{H}_2\text{O}_2$  can be noticed and is interpreted as due to the inability of  $\text{AH}_2$  donors to reduce  $\cdot\text{HO}_2$  radicals via an electron transfer reaction 17.

Difficulties similar to those mentioned above for the determination of the potential of the  $\cdot\text{HO}_2/\text{H}_2\text{O}_2$  couple are encountered for the  $\text{O}_2/\cdot\text{HO}_2$  couple. Based on the  $\text{p}K_a = 4.85$  for the  $\cdot\text{HO}_2$  radical, reaction 1, one can calculate that the potential for the  $\text{O}_2/\cdot\text{HO}_2$  will be  $\sim +0.3$  V higher than that of the  $\text{O}_2/\cdot\text{O}_2^-$  couple. Thus a  $E_k^{01}$  value of  $\sim +0.37$  V may be expected for the  $\text{O}_2/\cdot\text{HO}_2$  couple.

## Discussion

The titration method described above to follow the one-electron transfer reactions of  $\cdot\text{O}_2^-$  and  $\cdot\text{HO}_2$  radicals with electron acceptor, or electron donor, compounds gives kinetic potentials for the redox couples which appear to be

significant. While these potentials are not thermodynamic values, and are based on the known two-electron redox potentials of the compounds used, they do provide a scale for convenient comparison and prediction of the course of a chemical reaction.

Table III tabulates most of the known rate constants,  $k_{\text{obsd}}$ , for electron transfer processes of these species in aqueous solutions. Almost all these rates were determined previously<sup>24–35</sup> by various workers and using different techniques (including pulse radiolysis, flash photolysis, and esr).

The kinetic potential  $E_k^{01} = +0.07$  V for the  $\text{O}_2/\cdot\text{O}_2^-$  couple was determined above. Various calculated thermodynamic values of  $-0.59$  V,<sup>36</sup>  $-0.36$  V,<sup>37</sup> and  $-0.23$  V<sup>38</sup> for the same redox couples were reported. These values cannot be compared with the  $E_k^{01}$  value since different basis for reference were used. It can be shown, however, that the  $E_k^{01}$  value for this couple is in reasonably good agreement with data in the literature on the reactions of  $\cdot\text{O}_2^-$  and  $\cdot\text{HO}_2$ . The  $k_{\text{obsd}}$  values given in Table III for the reaction  $\cdot\text{O}_2^- + \text{A} \rightleftharpoons \text{O}_2 + \cdot\text{A}^-$  are all (except for cytochrome C, where steric barriers may affect the electron transfer process) in good agreement with the  $E_k^{01}$  and  $E^{01}$  potentials of  $\text{O}_2$  and A, respectively. Thus  $k_{\text{obsd}} \leq 5.6 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$  can be obtained. For and NADH,  $k_{\text{obsd}}$  is extremely slow as could have been predicted. It is clear that if one took the most positive thermodynamically calculated value for the  $\text{O}_2/\cdot\text{O}_2^-$  couple,  $E^{01} = -0.23$  V,<sup>38</sup> one could not explain the relatively low  $k_{\text{obsd}}$  values for menaquinone and duroquinone.

Similarly the  $k_{\text{obsd}}$  values for the reaction  $\cdot\text{A}^- + \text{O}_2 \rightleftharpoons \text{A} + \cdot\text{O}_2^-$  given in Table III and plotted in Figure 3 are in fairly good agreement with the  $E_k^{01}$  value for the  $\text{O}_2/\cdot\text{O}_2^-$  couple.

Much less information is available on the potential of the  $\text{O}_2/\text{H}_2\text{O}_2$  couple. A thermodynamic value of  $+0.90$  V has been calculated.<sup>37</sup> The  $E_k^{01}$  value for this couple  $\geq +0.36$  V.

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