BNL 28948

FORMATION AND DISAPPEARANCE OF SUPEROXIDE RADICALS IN AQUEOUS SOLUTIONS

by

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I. HISTORY

The existence of the radical HO_2 in gas-phase reactions was first ref. 1 proposed by Marshall[1] in 1926, and has since been found to play a prominent role in atmospheric chemistry. Its existence in aqueous ref. 2 solution was first proposed by Haber and Willstätter[2] in 1931 as an intermediate in the decomposition of hydrogen peroxide by catalase, supposed by them (incorrectly, as we now know) to be a chain reaction, carried by the radicals OH and HO_2:

$$\mathrm{Cat} + H_2O_2 \longrightarrow \mathrm{Cat\text{-}H} + HO_2 \qquad (0)$$

$$HO_2 + H_2O_2 \longrightarrow H_2O + O_2 + OH \qquad (1)$$

$$OH + H_2O_2 \longrightarrow H_2O + HO_2 \qquad (2)$$

ref. 3 Haber and Weiss[3] applied this concept to explain the reaction between hydrogen peroxide and ferrous iron:

$$Fe^{++} + H_2O_2 \longrightarrow Fe^{+++} + OH^- + OH \qquad (3)$$

followed by (2) and (1), or by

$$OH + Fe^{++} \longrightarrow Fe^{+++} + OH^- \qquad (4)$$

The fact that at high peroxide concentrations the amounts of peroxide consumed and oxygen formed are much greater than the amount of iron oxidized were explained as being due to reaction (1), which has become ref. 4 known as the "Haber-Weiss Reaction". Fenton[4] had shown long before that many organic acids and alcohols, ordinarily inert to peroxide, are rapidly oxidized by peroxide if ferrous salt is present. The formation of the very active oxidant OH explains this effect.

ref 5 In 1934, it was found[5] that the long-known crystalline superoxide of potassium, then called K_2O_4, was highly paramagnetic and must be regarded as composed of ions K^+ and O_2^-, the latter being paramagnetic ref 6 by virtue of its free-radical character. Weiss[6] then pointed out that this ion in aqueous solution would be the basic form of HO_2, and postulated different reactivities of the two forms to explain effects of pH on rates ref 7 of certain peroxide reactions. KO_2 had long been known[7] to react with water to form equal quantities of oxygen and peroxide, as one would expect from the disproportionation of O_2^- radicals:

$$O_2^- + O_2^- + H_2O \longrightarrow HO_2^- + O_2 + OH^- \qquad (5)$$

ref 8 where HO_2^- is the alkaline form of hydrogen peroxide. W. C. Bray[8] in 1938 pointed out that if reaction (1) was occurring, one would expect more oxygen than peroxide, and (5) must be the only important reaction of O_2^-, ref 9 and later experiments by Taube and Bray[9] verified this conclusion. Also ref 10 photochemical studies on H_2O_2[10] were inconsistent with an important role for reaction (1). In 1957, in a classical study in the field of reaction ref 11 kinetics in solution by Barb, Baxendale, George and Hargrave[11] the oxidation of ferrous and reduction of ferric ions by H_2O_2 was carefully studied. The formation of oxygen gas was conclusively shown to result from the reduction of ferric ions by HO_2 or H_2O_2, so that reaction (1) does not occur in any of the systems for which it was postulated. The subject is further discussed in a later section.

The development of nuclear energy during World War II required understanding of the effect of high-energy radiations on water and

aqueous solutions. Water is decomposed to yield free radicals H and OH,
solvated electrons and also molecules of hydrogen peroxide, which react
ref. 12 with the radicals to yield HO_2 or O_2^-.[12] However, until 1961 the
existence of the radical in aqueous solutions was only inferred from
reaction kinetics. Development of the method of electron spin resonance
ref. 13 spectroscopy allowed Saito and Bielski[13] to obtain in that year independent
confirmation of the existence of HO_2. Optical spectra of HO_2 were obtained
ref. 14, 15 in 1962 by flash photolysis[14] and in 1964 by pulse radiolysis,[15] which in
subsequent years has provided the most accurate data on the properties and
behavior of HO_2 and O_2^- in water solution.

The involvement of O_2^- in biological processes was shown in 1958
ref. 16 by Fridovich and Handler,[16] who reported evidence that O_2^- is formed
when xanthine oxidase acts to oxidize xanthine or other substrates. The
radical has since been shown to occur in many other enzymatic processes.

The latest chapter in the O_2^- story concerns the formation of
stable solutions of O_2^- in aprotic solvents--i.e. liquids such as dimethyl
sulfoxide (DMSO) which, though they may contain hydrogen, cannot donate
protons to other substances. Electrolytic reduction of dissolved oxygen
ref. 17 to O_2^- in DMSO was first shown in 1965.[17] A more convenient preparation
uses solutions in the aprotic solvent of a cyclic "crown" ether, which
complexes potassium ions and hence solubilizes potassium salts in organic
solvents. A 0.15 M solution of KO_2 with 0.3 M crown ether in DMSO
ref. 18 remains stable for many days.[18]

II. METHODS OF FORMATION OF AQUEOUS HO_2/HO_2^-

A. Radiolysis and Photolysis

Radiolysis of water produces free radicals OH and H and solvated electrons e^-_{aq}. If the water contains dissolved oxygen, O_2^- and HO_2 are formed:

$$e^-_{aq} + O_2 \longrightarrow O_2^- \qquad (6)$$

$$H + O_2 \longrightarrow HO_2 \qquad (7)$$

In the absence of a reducing agent, the HO_2/O_2^- will be destroyed by the OH:

$$OH + HO_2 \longrightarrow H_2O + O_2 \qquad (8)$$

If however the water contains dissolved hydrogen peroxide, all three of the primary radicals are converted to HO_2/O_2^-:

$$e^-_{aq} + H_2O_2 \longrightarrow OH + OH^- \qquad (9)$$

$$H + H_2O_2 \longrightarrow OH + H_2O \qquad (10)$$

$$OH + H_2O_2 \longrightarrow HO_2 + H_2O \qquad (11)$$

Whether the product appears in the form of HO_2 or O_2^- depends only on the pH of the solution, since the equilibrium $HO_2 \rightleftharpoons H^+ + O_2^-$ is attained in aqueous solution more rapidly than any other reaction of the radicals.

Since peroxide, especially in alkaline solution, can behave quite similarly to HO_2/O_2^-, studies on properties of this radical are better done in the absence of H_2O_2. A system has been found[19] which converts all primary radicals of water radiolysis to HO_2/O_2^- without using peroxide; it employs formate solutions containing oxygen. Reactions (6)

ref 19

and (7) convert e^-_{aq} and H, while the OH reacts with formate:

$$OH + HCOO^- \longrightarrow H_2O + CO_2^- \qquad (12)$$

$$CO_2^- + O_2 \longrightarrow O_2^- + CO_2 \qquad (13)$$

Radiolysis may be produced by X-rays or gamma rays, but much higher intensities are given by beams of high-energy electrons produced by machines. In the technique known as pulse radiolysis, an accelerator is induced to inject into a solution a pulse of electrons which, though it may last only a fraction of a microsecond, carries enough energy to produce chemical changes. The absorption spectra and lifetimes of transient species such as HO_2 are then followed optically by fast electronics. Once the apparatus is set up, properties and behavior of transient species (previously postulated only by inference from masses of laboriously obtained data on kinetics of overall chemical changes) can be quickly and accurately determined. Thus the turnover rate for superoxide dismutase has been determined far more accurately by pulse
ref. 20 radiolysis[20] than is possible by other methods.

Photolysis of hydrogen peroxide solutions by ultraviolet light of wavelength 3800–2000 Å results in splitting the H_2O_2 molecule into two OH radicals, which then yield HO_2/O_2^- by reaction (11). This method has not been used for preparation of O_2^-, because the high concentration of peroxide would interfere with study of O_2^- effects. However, light of shorter wavelength (less than 1800 Å) is intensely absorbed by water and produces H and OH radicals in good yields, which in oxygenated 0.02 M
ref. 21 formate solutions lead to O_2^- formation.[21] Inexpensive argon- or xenon-

filled lamps made with tubing of specially purified silica (Supersil) emit intensely in the appropriate wavelength range and provide a useful alternative to ionizing radiation for free radical studies.

fig. 1

At acid or neutral pH, the lifetime of HO_2/O_2^- is so short that it must be studied by pulse or fast-flow systems; but at high pH, its decay becomes slower (Figure 1). At pH 13 or above, solutions of 0.3 millimolar concentrations can readily be made by photolysis, X-rays or gamma rays. They will decay very little over several minutes after removal from the radiation and can be studied by conventional methods.

B. Electrolysis

ref. 22

ref. 23,24

ref. 25

Stable solutions of O_2^- are relatively easily prepared by electrolysis in liquid ammonia and in aprotic organic solvents,[17,22] but in water the reduction of oxygen normally goes all the way to H_2O_2 or even to H_2O. However, in alkaline solutions of surface-active materials, O_2 can be reduced to O_2^-,[23,24] which can be built up to concentrations as high as 1 mM.[25] It is thought that in the absence of surfactant, the O_2^- formed at the electrode surface remains there long enough to pick up a second electron from the cathode, together with a proton from the water to form hydrogen peroxide anion:

$$H_2O + O_2^- + e^- \longrightarrow HO_2^- + OH^- \tag{14}$$

In surfactant solutions the metal electrode adsorbs the non-polar hydrocarbon part of the molecule and becomes covered with a hydrocarbon layer. When a negative charge is applied to the metal, positive charge is induced on the polar ends of the surfactant layer. Oxygen molecules diffuse through this layer and accept an electron from the metal. The resulting O_2^- ions tend to be pulled away from the metal by the induced positive charge on the outside of the layer, and then become hydrated. The method seems to work better with a liquid mercury cathode than with solid metals. Successful O_2^- synthesis in aqueous solution by this method requires more skill with electrochemical and polarographic methodologies than most chemists have.

C. Mixing Nonaqueous Solutions into Water

So-called "stop-flow" systems in which flowing streams are mixed rapidly by mingling of high speed jets, and subsequent chemical reactions in the mixed stream are followed optically, have become widespread in recent years, with both streams usually being aqueous solutions. In such systems, well-designed mixers can achieve essentially complete mixing in one millisecond. With solvents of differing polarities, mixing is much slower, even under extreme turbulence. To mix dimethyl sulfoxide solution of O_2^- into water, the organic solution passed into a large excess of water through tiny holes; then the resulting suspension passed through two tangential mixers in series.[26] An optically homogeneous solution was obtained after 10 msec, allowing kinetic studies to be made of fast reactions of O_2^- in the aqueous phase.

ref 26

D. Chemical Reactions producing HO_2/O_2^-

The radical may be formed chemically by reduction of oxygen or oxidation of hydrogen peroxide. If the reducing or oxidizing agent acts by transference of only one electron at a time, HO_2/O_2^- will be present as a short-lived intermediate. It can be detected by adding a third reagent which is acted on by HO_2/O_2^- but not by the reagents used for producing the radical, or more directly by carrying out the reaction in, or flowing the reaction mixture through, an electron spin resonance (ESR) spectrometer. Because the ESR spectrum is sharper in frozen solutions, the reacting solution may be advantageously quickly frozen with liquid nitrogen, and the spectrum of the frozen solution then obtained.

Ceric sulfate, a powerful one-electron oxidizing agent in acid solution, rapidly oxidizes H_2O_2 to oxygen. When the reacting solution flowed through an ESR spectrometer, a spectrum typical of HO_2 was seen.[13] This method has been used to determine complex formation of HO_2 with a large number of metal ions.[27] In alkaline solutions, sodium periodate, $NaIO_4$, has been used with H_2O_2 to generate O_2^-.[28] There, the rapid freezing method was used to render the radical detectable by the ESR, since it is not seen by this method in water, nor in ice above -65°C.

ref. 27

ref. 28

In a related method, the Fenton reaction[4] may be used to generate HO_2. When ferrous or other strongly reducing metallic ions, such as titanous or vanadous, are mixed with excess hydrogen peroxide, reaction (3) is quickly followed by (2), and complexes of the resulting HO_2 were identified by ESR when various metal ions were added to the mixture.[27]

A peroxide solution, made to flow over metallic platinum, palladium or silver catalyst and then rapidly frozen showed the ESR spectrum of O_2^-; but the yield was much smaller than the amount of peroxide decomposed.[29]

ref. 29

A number of organic reducing agents can convert oxygen to O_2^- without enzymatic help. Tetraacetyl riboflavin reacts with EDTA under visible light to form the reduced flavin, the EDTA being oxidized to formaldehyde and other products. When this solution was mixed with oxygenated water in a flow system and rapidly frozen, the ESR spectrum of O_2^- was seen; cytochrome c was reduced by the O_2^-, and the radical ref. 30 was destroyed by superoxide dismutase.[30] The compounds 6-hydroxydopamine, 6-aminodopamine, 6,7-dihydroxytryptamine and dialuric acid, dissolved in O_2-free water, were found to take up oxygen rapidly; cytochrome c added ref. 31 to the solution was reduced, indicating the presence of O_2^-,[31] while this reduction was inhibited if superoxide dismutase was also added. In these reactions the O_2^- was not isolated.

ref. 32 It is claimed[32] that in the alkaline decomposition of concentrated (10–30%) aqueous hydrogen peroxide, concentrations of O_2^- approaching 2×10^{-3} M can be demonstrated by ESR with the fast-freezing method. Decomposition of H_2O_2 is fast when the pH equals the pK of H_2O_2 and the ref. 33,34 concentrations of H_2O_2 and HO_2^- are equal,[33,34] suggesting that the main reaction occurring is

$$H_2O_2 + HO_2^- \longrightarrow O_2 + H_2O + OH^- \tag{15}$$

O_2^- may be a product of a side reaction, possibly

$$H_2O_2 + 2HO_2^- \longrightarrow 2O_2^- + 2H_2O \tag{16}$$

Presence of O_2^- in concentrated alkaline peroxide could hardly be demonstrated any other way than by ESR, since the alkaline peroxide reacts similarly to O_2^- with the usual indicators, and its own optical absorption will cover up that of O_2^-.

E. Enzymatic Generation of O_2^-

Of the many enzymes which apparently generate superoxide radicals during enzymatic activity, only a few are of practical use in the study of O_2^-. These are the oxidoreductases which generate free O_2^- which can diffuse from the point of origin (active site) into the bulk of the solution for further reaction. The four proven enzymes to generate free ref. 35-38 O_2^- are: xanthine oxidase (xanthine: oxygen oxidoreductase 1.2.3.2),[35-38] ref. 39-42 aldehyde oxidase (aldehyde: oxygen oxidoreductase 1.2.3.1).[39-42] dihydroorotate oxidase (L-5.6-dihydroorotate: oxygen oxidoreductase ref. 43-45 1.3.3.1)[40,43-45] and NADPH-cytochrome reductase (NADPH: ferricytochrome ref. 46,47 oxidoreductase 1.6.2.4).[46,47] The enzyme most frequently used at present is xanthine oxidase because of its commercial availability in relatively pure form.

Xanthine oxidase is a very complex enzyme which contains 2 moles of flavin adenine dinucleotide (FAD), 8 moles of iron, 2 moles of molybdenum and labile sulfide groups. Its catalytic specificity is low both with respect to substrate oxidation (hypoxanthine, xanthine, pyrimidines, aldehydes, pterins, reduced nicotinamide adenine dinucleotide (NADH), etc.) and electron acceptors (oxygen, ferricyanide, artificial dyes). Although this is one of the most extensively studied enzymes, how it transfers electrons from donor to acceptor is not yet well understood.

In the standard laboratory method[35] for superoxide radical generation the preferred substrates are either xanthine or acetaldehyde which are oxidized to uric acid and acetic acid respectively. In the enzymatic process reduced xanthine oxidase apparently loses electrons by two different pathways, a univalent reaction yielding O_2^- (reaction 17) and a divalent reaction which leads directly to hydrogen peroxide formation (reaction 18):

$$\text{Enzyme-H}_2 + 2O_2 \longrightarrow \text{Enzyme} + 2H^+ + 2O_2^- \qquad (17)$$

$$\text{Enzyme-H}_2 + O_2 \longrightarrow \text{Enzyme} + H_2O_2 \qquad (18)$$

The percentage yield of superoxide radical in this system was found to vary from 20% to 100% depending upon pH, oxygen concentration and the turnover rate of the enzyme. In general raising the pH and oxygen concentration or lowering the turnover rate favors reaction (17).

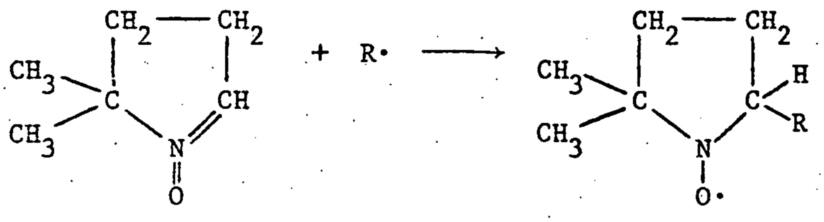
Although the xanthine oxidase system is a very useful source of superoxide radicals for steady state studies, great caution should be exercised in its use. The presence of hydrogen peroxide and inherent small amounts of metallic impurities in such enzyme systems can easily lead to Fenton-type reactions and hence to misinterpretation of observed results.

F. Photosensitization

Photosensitized oxidation has long been known. Many compounds, illuminated in the presence of air and a colored sensitizer (often a dye such as Rose Bengal (R.B.) or methylene blue) are oxidized or degraded. In the past, many such reactions have been attributed to the formation of energy-rich molecules of singlet oxygen $^1O_2$ by reaction of the light-excited sensitizer with ordinary triplet oxygen. It now seems that many such reactions involve O_2^- instead of, or in addition to, singlet oxygen. For instance, the chain oxidation of sulfite by air is ref.48 greatly speeded up by illumination in the presence of R.B.[48] The photoreaction is partially but not completely inhibited by superoxide dismutase (SOD), which destroys O_2^-, and also by a substance called Dabco, which is known to react rapidly with $^1O_2$. Thus the reaction is partially mediated by O_2^- and also by another pathway involving $^1O_2$. Oxidation of another compound, 2,3-diphenyl-p-dioxene, was inhibited only by Dabco not by SOD so that in that case no O_2^- was involved.

A different type of sensitizer is represented by the bipyridyl-complexed divalent ruthenium ion, $Ru(bipy)_3^{2+}$. Illuminated in acetonitrile solution, this accepts an electron from any good electron donor (E.D.) which may be present to form the unstable reduced ion, $Ru(bipy)_3^+$, seen ref.49 as a transient spectrum along with the spectrum of the $(E.D.)^+$ ion.[49] When oxygen was present, the spectrum of $Ru(bipy)_3^+$ did not appear, only $(E.D.)^+$, and it must be assumed that O_2 oxidizes Ru back to its original form, while taking on an electron to become O_2^-. The ion $(E.D.)^+$ disappears by a rapid second-order process, obviously $O_2^- + (E.D.)^+$, so no net chemical change occurs in the end.

More direct evidence for photosensitized O_2^- formation is provided by the method of "spin trapping". Certain nitroxides, such as 5,5'-dimethyl-1-pyrroline-1-oxide (DMPO) readily add a free radical R· to form a very stable nitroxyl radical:



(19)

The ESR spectrum of the product radical then provides information on the nature of R•. The O_2^- adduct of DMPO appears on illumination of its solution in aqueous H_2O_2.[50] By this method it was proved[51] that O_2^- forms when a suspension in water or acetonitrile of a semiconductor, such as cadmium sulfide or phthalocyanine, is illuminated. Solutions of metalloporphyrins in aprotic solvents were also shown by spin-trapping to give a good yield of O_2^-,[52] but in these cases there was also evidence for large yields of $^1O_2$.

ref. 50, 51

ref. 52

One aromatic photosensitizing molecule, 9,10-dicyanoanthracene (DCA), has been reported[53] which appears to operate through O_2^- exclusively, with essentially no yield of $^1O_2$. However, the existence of free O_2^- was not established. The E.D.'s used all quenched the DCA fluorescence very efficiently, and the initial step was thought to be complex formation with partial or complete charge transfer in the complex:

ref. 53

$$\mathrm{DCA} \xrightarrow{h\nu} \mathrm{DCA}^* \xrightarrow{\mathrm{ED}} [\mathrm{DCA}^- - \mathrm{E.D.}^+] \xrightarrow{O_2} \left[\begin{matrix}\mathrm{DCA} - \mathrm{E.D.}^+ \\ O_2^-\end{matrix}\right] \longrightarrow \mathrm{DCA} + \text{products} \qquad (20)$$

Thus the whole process could occur in the vicinity of the excited DCA molecule without the O_2^- ever becoming free. The observed products included benzaldehyde from stilbene, benzophenone from tetraphenylene, and sulfoxides from diethyl and diphenyl sulfides, which could not be formed from $^1O_2$ in the observed yields.

III. PROPERTIES OF HO_2/O_2^- IN AQUEOUS SOLUTION

A. Spontaneous Dismutation Rates, pK and Absorption Spectra

The pulse radiolysis method is especially well suited to determining lifetimes of transient species such as HO_2/O_2^-. An early paper[54] showed that these species have a strong optical absorption in the ultraviolet, more intense in neutral than in acid solutions, which can be used to trace the course of the decay in time. In this work, the decay was mainly kinetically second order at all pH's studied, but also appeared to have a smaller first-order component, which could be due to catalysis by impurities. The study was then carried on at another laboratory, with improved pulse radiolysis techniques and sample purification.[19] Purely second-order decays were found from pH 0 to 8, but a slow first-order component, ascribed to impurities appeared in alkaline solutions. The best spectra of HO_2 and O_2^- available at present are shown in Figure 2.[55] The spectrum seen near the pK is intermediate, and depends on the ratio of the two components present, so that from the change in the spectra as pH is varied, the pK can be determined.

ref.54

ref.55

The decay rate, nearly constant at pH less than 2, rises to a maximum near the pK, where the two forms are of equal concentration, and then becomes much smaller in alkali (Figure 1). This behavior is explained by the necessity of having at least two different bimolecular disproportionation reactions:

$$HO_2 + HO_2 \longrightarrow H_2O_2 + O_2 \qquad (21)$$

$$HO_2 + O_2^- \longrightarrow HO_2^- + O_2 \qquad (22)$$

HO_2^- is the basic form of hydrogen peroxide. The maximum in the curve of rate vs. pH occurs because (22) is faster than (21). Three constants are required to fit the curve--k_{21}, k_{22} and the pK. Thus the data of these experiments provide two independent methods--kinetic and spectroscopic--for determination of the pK of HO_2.

The third possibility for a disproportionation reaction is, of course

$$O_2^- + O_2^- + H_2O \longrightarrow HO_2^- + O_2 + OH^- \qquad (5)$$

This reaction was shown[19,54] to be much slower than (21) or (22), but its rate could not be determined because of the occurrence of the impurity-catalyzed first-order disappearance of O_2^-. The work was ref. 56 therefore repeated[56] with specially purified reagents, and with the complexing agent EDTA present to sequester possibly catalytic metal ions. The result was that reaction (5) does not occur at all ($k_5 < 0.3\ M^{-1}\ s^{-1}$) and a 10^{-4} M solution of O_2^- at pH 13 has an initial half-life of about 160 minutes.

Recently[55] a careful redetermination of the optical absorption fig. 1 coefficients led to the spectrum shown in Fig. 1 and the recalculated disproportionation rates plotted in Fig. 2. The pK_{HO_2} is 4.69 ± 0.08 (about 0.2 less than the often-quoted earlier value); $k_{21} = 8.6 \times 10^5\ M^{-1}\ s^{-1}$ and $k_{22} = 1.02 \times 10^8\ M^{-1}\ s^{-1}$.

B. Catalyzed Dismutation

Especially in solutions above pH 7, the catalyzed dismutation is thus more important practically than the spontaneous reaction, and has been extensively studied. The most famous catalysts are of course the superoxide dismutases, which are proteins containing copper, manganese or iron. The rate of the bimolecular reaction between O_2^- and enzyme, leading to destruction of O_2^-, has been determined by pulse radiolysis to be $1.3 \times 10^9\ M^{-1}\ s^{-1}$ per copper atom for bovine erythrocuprein[20,57,58] and coincidentally the same number for a manganese-containing enzyme from E. coli.[59] The erythrocuprein contains a zinc atom as well as copper, which can be replaced by cobalt; this replacement had no effect on the reaction with O_2^-.[60]

ref. 57, 58

ref. 59

ref. 60

A large number of copper compounds have been tested for the rate at which they react in aqueous solution with O_2^- to catalyze its dismutation; the results are shown in Table 1. It is noteworthy that plain uncomplexed aqueous copper ion is an even more effective "dismutase" than any enzyme. Soluble copper complexes may show catalytic activity, ranging unpredictably from very high to zero; the behavior towards O_2^- depends on the detailed arrangement of ligands in a way which is not well understood. In the case of copper-histidine complexes, six different complexes have been characterized with different Cu/Hist ratios and degrees of protonation; of these, one ($CuHist_2H^{3+}$) reacts rapidly with O_2^- ($k = 3.4 \times 10^8\ M^{-1}\ s^{-1}$) but the other five do not react noticeably at all.[61] One doubts that the complexes with all the other ligands listed in Table I have been as thoroughly characterized as those with histidine, and the numbers given may in some cases be average values for whatever complexes may have been present under the particular conditions of the experiments.

Table 1

ref. 61

Although it had been suggested in the past that the copper-catalyzed

ref. 64,65 two-step dismutation might involve a copper-superoxide radical complex,[18,64,65] overall the mechanism was believed to cycle back and forth between Cu^{++} and Cu^{+}:

$$Cu^{++} + O_2^{-} \longrightarrow CuO_2^{+} \longrightarrow Cu^{+} + O_2 \qquad (23)$$

$$Cu^{+} + O_2^{-} + 2H^{+} \longrightarrow Cu^{++} + H_2O_2 \qquad (24)$$

ref. 63 More recently[63] it was shown, by mixing a large excess of Cu^{++} with HO_2/O_2^{-} (Cu^{++}/HO_2 10^3-10^4) that indeed one or more complexes are formed, which absorb strongly in the UV region:

$$Cu^{++} + O_2^{-} \longrightarrow CuO_2^{+} \qquad (25)$$

$$CuO_2^{+} + Cu^{++} \longrightarrow (Cu^{++}\cdots O_2^{-}\cdots Cu^{++}) \qquad (26)$$

This system is extremely complex since the complexes can be present in various states of protonation on which is superimposed the equilibrium $HO_2 \rightleftharpoons O_2^{-} + H^{+}$.

As in the spontaneous disproportionation of superoxide radicals, where the controlling step is reaction (22) between HO_2 and O_2^{-}, it was

ref. 66 suggested[66] that in the copper catalyzed dismutation the Cu^{++}-cation plays the same role as the proton in HO_2:

$$Cu^{++} + O_2^{-} \longrightarrow CuO_2^{+} \qquad (25)$$

$$CuO_2^{+} + O_2^{-} + 2H_2O \longrightarrow Cu^{++} + H_2O_2 + O_2 + 2OH^{-} \qquad (27)$$

ref. 67 While complex formation between O_2^{-} and metal cations (Fe^{++};[11,67]

ref. 68-72 Mn^{++};[68,69] Ba^{++}, Ca^{++};[70] Ni^{++}, Cr^{++};[71,72] V^{5+}, Ti^{4+}, Ce^{3+}, Zr^{4+}, Th^{4+}, U^{6+}, Mo^{6+}[27])

is well documented, convincing evidence for a superoxide dismutase-O_2^- complex is lacking.

ref. 73

Although copper-EDTA complexes do not react with O_2^-, iron-EDTA complexes do. The reactions were studied[73] in a stopped-flow system in which a small volume of a DMSO solution of O_2^- was mixed with a large volume of very dilute aqueous Fe^{++}-EDTA or Fe^{+++}-EDTA complex. The reaction formed the same purple peroxo complex $[Fe^{+++}\text{-EDTA-}O_2]^{3-}$ that is formed from hydrogen peroxide and Fe^{++}-EDTA at high alkalinity. The redox reactions which occurred at pH 9.7 resulted in the disproportionation of O_2^-:

$$[Fe^{++} - \text{EDTA}]^{2-} + O_2^- \longrightarrow [Fe^{+++} - \text{EDTA} - O_2]^{3-} \quad (28)$$

$$[Fe^{+++} - \text{EDTA} - O_2]^{3-} + H^+ \rightleftharpoons [Fe^{+++} - \text{EDTA}]^- + HO_2 \quad (29)$$

$$[Fe^{+++} - \text{EDTA}]^- + O_2^- \longrightarrow [Fe^{++} - \text{EDTA}]^{2-} + O_2 \quad (30)$$

However, reaction (29) requires seconds and the induced disproportionation rate is barely faster than the spontaneous disproportionation at this pH, so Fe-EDTA is not really much of a catalyst.

In contrast to the much-studied copper situation, we have found no work on the reactions of O_2^- with other forms of iron in alkaline solution. In acid solution, the reactions of Fe^{++} and Fe^{+++} with HO_2 and H_2O_2 have been thoroughly worked out.[11,67] In biological systems, complexed ferrous iron may be important in converting hydrogen peroxide by the Fenton reaction to the very energy-rich oxidant OH.

Manganese, the other metal found in some superoxide dismutases, has been relatively little studied with respect to its reactions with O_2^-.

The usual form in aqueous solution, Mn^{++}, reacts with O_2^- in the equilibrium reaction[68,69]

$$Mn^{++} + O_2^- \rightleftharpoons MnO_2^+ \qquad (31)$$

This complex does not go to any higher oxidation state of Mn, and Mn^{++}_{aq} does not catalyze dismutation of O_2^-. However, in Mn-containing dismutases the metal is normally in the trivalent state, but can be reduced to the divalent state without loss of enzymatic activity.[74] Mn^{+++} is stable only in organic complexes. As a redox model for the action of the enzyme, complexes of Mn with 8-quinolinol were reacted with O_2^- in dimethyl sulfoxide solution.[75] A bewildering variety of complexes appeared to be involved in the reactions, but oxidation and reduction of the manganese surely occurred and dismutation of O_2^- resulted.

ref. 74

ref. 75

C. Thermodynamics

The free energy relationships of the stable compounds of hydrogen and oxygen have long been known very accurately.[76] To bring the radicals HO_2 and O_2^- into this picture, and establish their standard electrode potentials required a determination of the electrode potential of O_2^-, corresponding to the half-reaction

ref. 76

$$O_2 + e^- \longrightarrow O_2^- \qquad (32)$$

Good electrochemical determinations have been made of this quantity,[25,77] but the most accurate method[78] measures the equilibrium between O_2, O_2^-, and quinone-semiquinone couple of known potential:

ref. 77

ref. 78

$$H^+ + O_2^- + Q \rightleftharpoons O_2 + QH\cdot \qquad (33)$$

From the equilibrium constant, the standard free energy change is obtained. Standard states of most solutes are unit molality in water, but two usages can be found in the case of oxygen: its free energy can be related to a 1 molal solution of the gas, or to the gas at atmospheric pressure, which corresponds to a 1.25×10^{-3} M aqueous solution. Here we use the latter system. In Table 2 we list the standard free energy of formation from the elements (H_2 and O_2) of various compounds in aqueous solution at 25°C. From the values in this table, together with the pK's of HO_2 (4.7) and H_2O_2 (11.6) we obtain the electrode potentials listed in Table 3.

table 2

table 3

D. The So-Called Haber-Weiss Reaction

There is no question that the great papers of Haber and Weiss[3] marked the opening of the whole field of free radical chemistry in aqueous solution, and these men deserve to be known as the fathers of this field of chemistry. Of course, it is too much to expect even of these brilliant chemists that in the very beginning of the subject they should get every detail of their mechanisms right; and, as described at the beginning of this chapter, in their mechanisms for the iron-peroxide reaction, they proposed as a single step the reaction between HO_2 and H_2O_2

$$HO_2 + H_2O_2 \longrightarrow H_2O + O_2 + OH \qquad (1)$$

which was later shown to occur in two steps

$$HO_2 + Fe^{+++} \longrightarrow O_2 + Fe^{++} + H^+ \qquad (34)$$

$$Fe^{++} + H_2O_2 \longrightarrow Fe^{+++} + OH + OH^- \qquad (3)$$

Reaction (3) is of course the long-known Fenton reaction,[4] and this name is used to describe the generation of OH radicals from hydrogen peroxide by metal-containing reducing agents generally.

In more recent years, many biochemists have noticed that O_2^- and H_2O_2, generated together enzymatically, led to oxidative effects suggesting formation of a powerful active oxidizing agent, reasonably assumed to be the OH radical. Not being aware of all the evidence amassed over the decades by physical chemists against reaction (1), they attributed the formation of OH to this reaction and dubbed it "Haber-Weiss". By now the facts are obvious to all,[80] first, that reaction (1) does not occur spontaneously, second, that these biological effects suggesting OH radical production occurred in the presence of metal-containing enzymes and should be attributed to reactions of the Fenton type.

ref. 80

Thus the controversy that has arisen is not over a matter of substance, but merely over a name. Especially those of us who knew and admired Joseph Weiss for his many fine achievements in the development of free radical chemistry hate to see his name immortalized through attachment to an early proposal which proved to be a mistake. Surely, if the reaction generating OH radicals (or a similar strong oxidant) must be referred to by name, it should be given its proper designation, by which it was known long before Haber and Weiss published their theory; namely, the Fenton Reaction.

Table 1

Rate constants for reaction of O_2^- with copper compounds in aqueous solution leading to catalytic dismutation of O_2^-, calculated on the basis of the number of copper atoms present.

Compound	pH	k, M^{-1} s^{-1}	Ref.
Cu_{aq}^{++}	2	2.7×10^9	(58)
Bovine erythrocuprein (SOD)	7-9	1.3×10^9	(20,57,58)
Cu_2(Indomethacin)$_4$	7	6.0×10^9	(62)
Cu(Tyrosine)$_2$		1.3×10^9	(60)
$Cu(HCOO)^+$ (a)	6	1.7×10^9	(63)
$Cu(HCOO)_2$	7	3.0×10^8	(63)
$Cu(HCOO)_3^-$	7	8.0×10^8	(63)
$Cu(HCOO)_4^{2-}$	8	4.0×10^8	(63)
$Cu(NH_3)$ complexes	∿ 10	$0.2\text{-}2.2 \times 10^9$	(63)
$CuSO_4$	6	5.0×10^8	(63)
$CuCl_3^-$	7	7.0×10^8	(63)
Cu(Histidine-H)$^{3+}$		3.4×10^8	(61)
Cu(Lysine)$_2$		6.0×10^8	(58)
Cu(Glycine)$_2$	8	2.0×10^6	(63)
Cu(Glycylglycine)$_2$	7	2.0×10^7	(63)
Cu(Glycylhistidine)$_2$		3.0×10^8	(58)
Cu(Glycylhistidylleucine)$_2$		2.0×10^8	(58)
Cu(Alanine)$_2$	7	3.0×10^6	(63)
Cu-glutamate	7	1.0×10^6	(63)
Cu(Hydroxyproline)	8	1.0×10^6	(63)
Cu(Methionine)$_x$	7-8	$5.0\text{-}8.0 \times 10^6$	(63)

(a) Ionic strength 2.0 was maintained in the formate studies. Values for zero ionic strength may differ by an order of magnitude.

Table 2

Standard free energies of formation ΔG(f) for various species in aqueous solution at 25°C

Compound	ΔG(f), kcal/mole	Ref.
H_2O (liquid)	−56.687	(76)
OH^-	−37.594	(76)
H_2O_2	−32.05	(76)
HO_2^-	−16.1	(76)
HO_2	+ 1.2	(25)
OH	+ 4.5	(79)
O_2^-	+ 7.6	(78)

Table 3

Standard electrode potential for some redox reactions at 25°C relative to the hydrogen electrode. Standard states are, for H_2 and O_2, gas at 1 atm.; H_2O (liquid); other substances, unit molality.

Reaction	E^o (volts)
$OH + e^- \longrightarrow OH^-$	+ 1.83
$O_2^- + e^- + 2H^+ \longrightarrow H_2O_2$	+ 1.73
$HO_2 + e^- + H^+ \longrightarrow H_2O_2$	+ 1.45
$O_2^- + e^- + H^+ \longrightarrow HO_2^-$	+ 1.03
$O_2 + e^- + 2H^+ \longrightarrow H_2O_2$	+ 0.695
$O_2 + 2e^- + H^+ \longrightarrow HO_2^-$	+ 0.35
$e^- + H^+ \longrightarrow \frac{1}{2}H_2$	0.000
$O_2 + e^- + H^+ \longrightarrow HO_2$	- 0.05
$O_2 + e^- \longrightarrow O_2^-$	- 0.33

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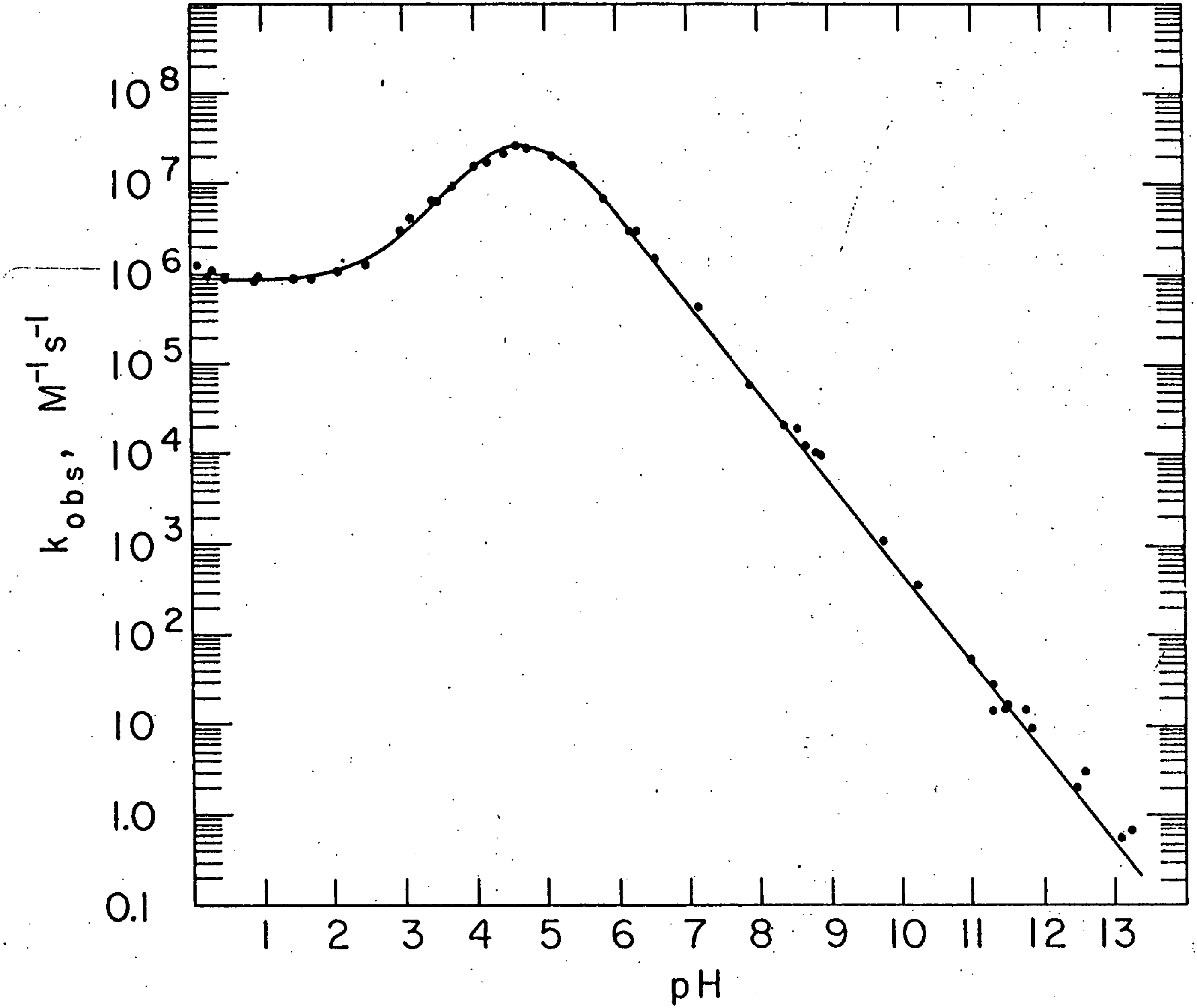
Figure Captions

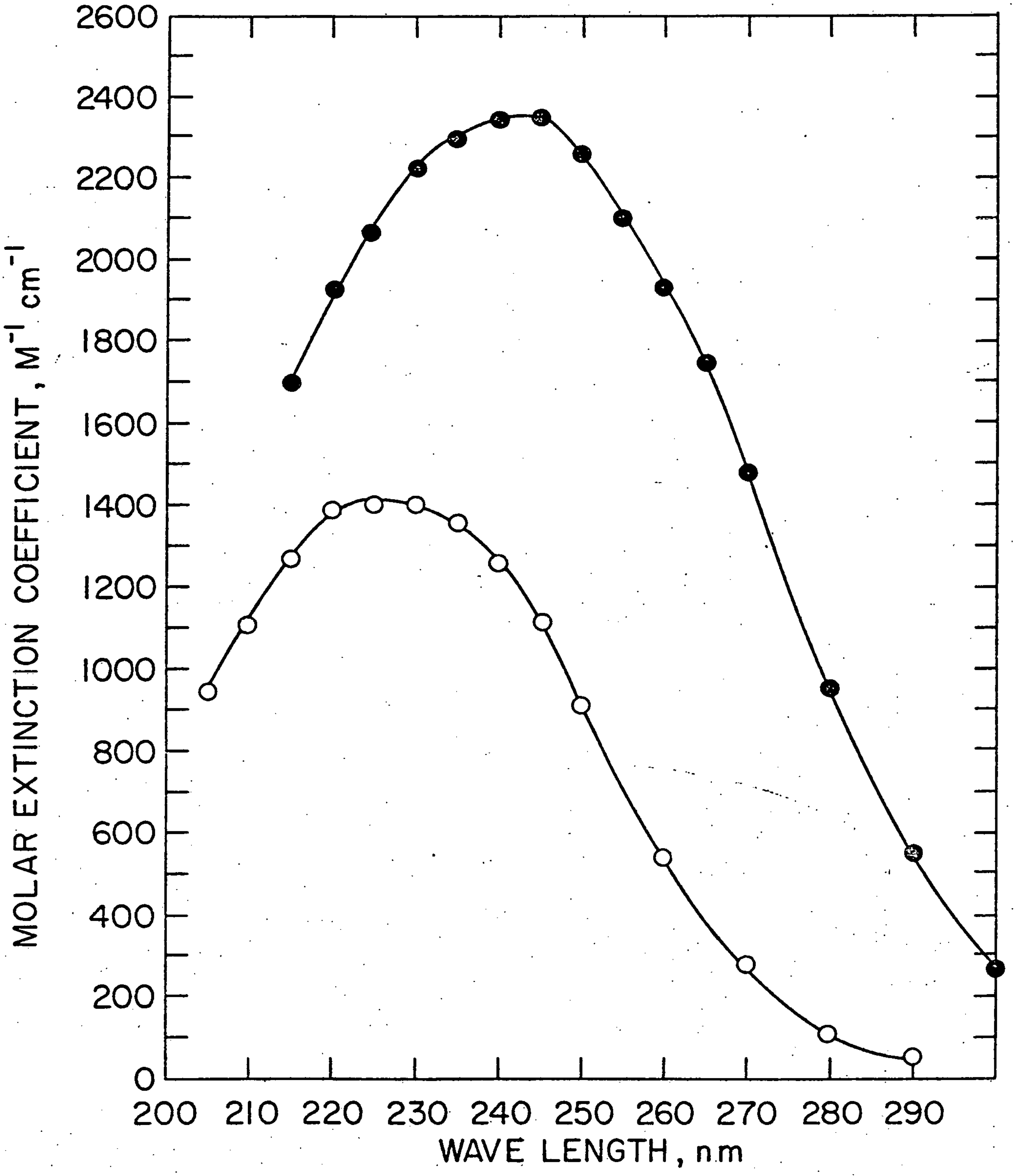
Figure 1. Rate constant for spontaneous second-order decay of HO_2/O_2^-.[55]

Figure 2. Absorption spectra of O_2^- at pH 7 (upper curve) and HO_2 at pH 2 (lower curve).[55]

Figure 1



10^8
10^7
10^6
10^5
10^4
10^3
10^2
10
1.0
0.1
k_{obs}, $M^{-1}s^{-1}$
1
2
3
4
5
6
7
8
9
10
11
12
13
pH



MOLAR EXTINCTION COEFFICIENT, M^{-1} cm^{-1}
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
200
210
220
230
240
250
260
270
280
290
WAVE LENGTH, nm

Figure 2