Redox Potentials of Free Radicals. I. Simple Organic Radicals

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Abstract: The redox potentials of some free radicals in aqueous solution have been determined using a new method. This method is described in detail and is based on the electron transfer properties and characteristics of the donor radical ·RH to a range of acceptor molecules A. The reaction $\cdot RH + A \rightleftharpoons R + \cdot A^- + H^+$ occurs at an appreciable rate whenever the redox potential $E^{\circ}{}^1$ (V, at pH 7.0, ~25°) of A is higher than that of ·RH. The percentage efficiency of this electron transfer process is determined for a large number of acceptors by monitoring the formation of the $\cdot A^-$ radicals at the appropriate pH and wavelength. The fast reaction technique of pulse radiolysis and kinetic absorption spectrophotometry has been used to follow these reactions. Typical "titration" type curves are obtained when plotting the percentage efficiency *vs.* $E^{\circ}{}^1$ of the acceptors. From the midpoint of such curves, the redox potentials of a number of organic free radicals have been determined. Various radicals derived from aliphatic alcohols, sugars, lactic acid, glycolic acid, lactamide, glycolamide, oxalacetic acid, glycine, and glycine anhydride have been produced and their potentials derived. These radicals are known to undergo acid–base reactions, and the variations of the $E^{\circ}{}^1$ as a function of the acid or base forms of the radicals were studied. In all cases, the ionized (basic) forms of the radicals have significantly lower redox potentials than the acid forms of the radicals, making these radicals more powerful reducing agents. Some aspects of the mechanisms and kinetics of free radical reactions in the literature are discussed and can be rationalized on the basis of the determined redox potentials of these free radicals.

The chemistry of free radicals in solution has developed very rapidly during the last 10–15 years. Considerable knowledge and information have been obtained on the reaction mechanisms and chemical dynamics of free radicals, and a very large number and a wide range of organic and inorganic free radicals have been produced and studied. Much of the detailed and quantitative basis of these reactions has been obtained using fast reaction techniques (rapid-mix flows, pulse radiolysis, flash photolysis), electron spin resonance spectroscopy, and polarography. While all this information has been most valuable, it has developed in a rather empirical manner and no cohesive interpretation is presently available which can explain or predict the course of a free radical chemical reaction or its reaction rate constant. Clearly, this is a challenge to theoretical chemists and spectroscopists.

One of a number of gaps in our understanding of these reactions is a knowledge of the redox potential of free radicals. This may be of considerable importance in affecting the course of chemical reactions. Very little work has been done in determining the redox potential of free radicals ($E^{\circ}{}^1$, pH 7.0, ~25°), due to the experimental difficulties encountered in producing a free radical and simultaneously measuring its $E^{\circ}{}^1$ value before it decays. Polarography and electrochemistry have contributed[2] in this respect. However, it is limited to relatively stable long-lived species, usually radical anions, produced in "unreactive" aprotic solvents or radicals stabilized in very acidic or very alkaline solutions. A combination of polarography and pulse radiolysis has been developed[3] with a time resolution of $<10^{-4}$ sec. To this valuable technique has been added an alternating current conductivity method[4] in conjunction with pulse radiolysis, having a response time of ≤ 2 μsec. These techniques, however, have certain problems and limitations (see ref 5 and below).

A new and novel approach to the determination of the redox potentials of free radicals has been developed and is described below. Basically, this method depends on the electron transfer properties[6-13] of free radicals (·RH) to a series of acceptors (A) whose redox potentials are known.

$$\cdot RH + A \rightleftharpoons R + \cdot A^- + H^+ \qquad (1)$$

Preliminary results obtained using this method have been published.[14] This method also affords the possibility of determining the redox potentials of various acid and base forms of free radicals[15] produced over a wide pH range in aqueous solutions.

Experimental Section

The technique of pulse radiolysis and kinetic absorption spectrophotometry was used to produce the free radicals examined in this work and follow their one-electron redox reactions. The experimental details have already been described.[16,17] Single pulses of ~2.3-MeV electrons and ~30-nsec duration were used and provided by the Febetron (Field Emission Corp.) 705 pulsed radiation source.

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The substrates (RH_2) giving rise to the free radicals were dissolved in water. The radiation chemistry of water produces e_{aq}^-, OH, and H atoms

$$H_2O \xrightarrow{\sim\sim} e_{aq}^- (2.8), OH (2.8), \text{ and } H (0.6)$$

where the values in parentheses are G values (yields of radicals produced per 100 eV of energy absorbed by the aqueous solution). The hydrated electron can be converted to OH radicals on saturation of the solution with N_2O ($\sim 2.5 \times 10^{-2}$ M)

$$e_{aq}^- + N_2O \rightarrow N_2 + OH + OH^- \quad (2)$$

where $k_2 = 8.7 \times 10^9$ M^{-1} sec^{-1} (ref 18). All the free radicals examined were produced (except for one radical, see below) by reaction with hydroxyl radicals, according to reaction 3. Most of

$$RH_2 + OH \longrightarrow \cdot RH + H_2O \quad (3)$$

the H atoms also reacted with the substrate to produce similar radicals.

$$H + RH_2 \longrightarrow \cdot RH + H_2 \quad (4)$$

The experimental conditions were adjusted such that (a) all the e_{aq}^- reacted with N_2O and none with the substrates, (b) all the OH radicals were scavenged by the substrates, and (c) very low radiation doses were used ($\sim$0.15–1.20 krads/pulse) in order to reduce the second-order radical–radical decay of the radicals (reaction 5) and allow the radicals ($\geq 90\%$) to react with the acceptors A, according to reaction 1.

$$\cdot RH + \cdot RH \longrightarrow \text{products} \quad (5)$$

The decay rates k_5 had previously been determined for all the radicals studied here (see more below).

The chemicals used were the highest research grade commercially available and were obtained from J. T. Baker, Eastman Chemicals, Aldrich, Calbiochem, Mallinckrodt, and K & K Laboratories. The solutions were prepared just prior to their irradiation, and the pH was adjusted using perchloric acid, potassium hydroxide, and $\sim$1 mM phosphate and tetraborate buffers.

Determination of Redox Potentials. The method developed to determine the redox potentials of free radicals in aqueous solution is based on the electron transfer properties of the free radical, reaction 1. Such a transfer of electrons takes place provided the redox potential of the acceptor A is higher than that of the donor radical.

The technique is based on the following two half-cell reactions (for this purpose the radical is expressed as $\cdot R^-$)

$$\cdot R^- \rightleftharpoons R + e^- \quad (A)$$

$$A + e^- \rightleftharpoons \cdot A^- \quad (B)$$

and the overall reaction is

$$\cdot R^- + A \rightleftharpoons R + \cdot A^- \quad (C)$$

From the Nernst equation

$$E = E^\circ + \frac{RT}{nF} \ln \frac{[Ox]}{[Red]}$$

Two equations can be considered for these systems (at 25°).

$$E = E^\circ_A + \frac{0.059}{n} \log \frac{[A]}{[\cdot A^-]} \quad (D)$$

$$E = E^\circ_{\cdot R^-} + \frac{0.059}{n} \log \frac{[\cdot R^-]}{[R]} \quad (E)$$

If X = the amount of A reacted and C_i = the initial concentration of A and of $\cdot R^-$ radicals produced by pulse radiolysis, then

$$E = E^\circ_A + \frac{0.059}{n} \log \frac{C_A - X}{X}$$

$$E = E^\circ_{\cdot R^-} + \frac{0.059}{n} \log \frac{C_{\cdot R^-} - X}{X}$$

and if α = the fraction of radicals which have reacted

$$\alpha = X/C_{\cdot R^-}$$

then

$$E = E^\circ_A + \frac{0.059}{n} \log \frac{C_A - \alpha C_{\cdot R^-}}{\alpha C_{\cdot R^-}}$$

$$E = E^\circ_{\cdot R^-} + \frac{0.059}{n} \log \frac{1 - \alpha}{\alpha}$$

Under equilibrium conditions

$$E^\circ_A + \frac{0.059}{n} \log \frac{C_A - \alpha C_{\cdot R^-}}{\alpha C_{\cdot R^-}} = -\left(E^\circ_{\cdot R^-} + \frac{0.059}{n} \log \frac{1 - \alpha}{\alpha}\right)$$

At midpoint (50% electron transfer), $\alpha = 0.5$ and

$$-E^\circ_{\cdot R^-} = E^\circ_A + \frac{0.059}{n} \log \frac{2C_A - C_{\cdot R^-}}{C_{\cdot R^-}} \quad (F)$$

The initial concentrations of C_A and $C_{\cdot R^-}$ were chosen (see also below) such that $C_A/C_{\cdot R^-}$ equals $\sim$12–15, in all cases. Equation F can be reduced approximately (when $n = 1$) to

$$-E^\circ_{\cdot R^-} = E^\circ_A + 0.059 \log 27 = E^\circ_A + 0.085 \quad (G)$$

The efficiency of transfer of an electron, expressed as percentage, is dependent on the difference between the redox potentials of the donor and the acceptor. A plot of the percentage efficiency of electron transfer against the redox potential of the acceptors gives typical "titration" curves, and from the midpoint (50% transfer) the E° [1] of the free radical was derived.

The transfer of an electron to A, reaction 1, was monitored in two ways. The first was (a) by following the formation of $\cdot A^-$ (or the protonated form $\cdot A^-$–H^+, depending on the pK_a of the radical produced from the acceptor) at the appropriate pH and wavelength where $\cdot A^-$ absorbs. Based on the determined extinction coefficient of $\cdot A^-$ (see more below), the percentage of electron transfer can readily be calculated. This method allows one to determine, in addition, the rate constant for the electron transfer process. Indeed, the rate of formation of $\cdot A^-$ is pseudo-first order dependent on the concentration of the acceptor. This is checked routinely. (b) The second way was by following the disappearance or "bleaching" of the acceptor (usually dyes, in this case) at the appropriate wavelength. Again, based on the extinction coefficients of the dyes at that wavelength and pH, the efficiency of electron transfer to form the dye semiquinone radical is readily determined.

In order to minimize errors and improve the accuracy of the results, the absorbance due to 100% formation of $\cdot A^-$, or 100% bleaching of the dye, was determined *in all cases* immediately previous to carrying out the experiment. This was obtained by reacting the acceptors with e_{aq}^-

$$e_{aq}^- + A \longrightarrow \cdot A^- \quad (6)$$

in solutions containing the same concentration of A, 1.0 M *t*-BuOH (to scavenge the OH radicals), and 1 atm of argon. The same pH, radiation dose, monitored wavelength, and monochromator slit widths are used. In this way, a direct determination of the extinction coefficients equivalent to 100% efficiency of formation of $\cdot A^-$, or disappearance of A, made it possible to obtain accurate and reliable results. At the time scale the experiments were carried out, the decay of $\cdot A^-$ radicals was negligible.

Table I shows the list of acceptors used, their redox potentials,[19–21] the wavelengths monitored, and the pK_a of the radicals or radical

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Table I. List of Acceptors Used

No.	Acceptor	$E^{\circ 1}$, V[a]	λ (nm)[b] monitored	pK_a (radical)
1.	Acetophenone	−1.290	445	9.9
2.	Benzophenone	−1.000	545, 615	9.25
3.	3-Benzoylpyridine	−0.750	530	9.2
4.	Fluorescein	−0.572	500*	
5.	Rhodamine B	−0.542	520*	
6.	Eosin Y	−0.500	520*	
7.	Methyl viologen	−0.446	385	
8.	Basic fuchsine	−0.430	520	
9.	Benzil	−0.395	545	5.5
10.	Crystal violet	−0.357	525*	
11.	NAD^+	−0.320	400	
12.	Safranine T	−0.289	520*	
13.	9,10-Anthraquinone	−0.266	400	5.3
14.	Phenosafranine	−0.254	520*	
15.	9,10-Anthraquinone-2-sulfonate	−0.250	400	3.25
16.	Riboflavine	−0.208	560	8.3
17.	9,10-Anthraquinone-2,6-disulfonate	−0.184	385, 400	3.2
18.	2-Hydroxy-1,4-naphthoquinone	−0.139	370, 390	4.7
19.	Indigodisulfonate	−0.125	610*	
20.	Indigotetrasulfonate	−0.046	610*	
21.	Menaquinone	+0.002	370, 400	4.5
22.	Methylene Blue	+0.011	580*	
23.	Thionine (pH 8)	+0.031	600*	
24.	Duroquinone	+0.068	445	5.1
25.	Indophenol (pH 9)	+0.089	610*	
26.	1,4-Naphthoquinone-2-sulfonate	+0.118	380, 400	4.2
27.	2,5-Dimethyl-*p*-benzoquinone	+0.176	415, 440	4.6
28.	2,5-Dichloroindophenol	+0.217	600*	
29.	*p*-Benzoquinone	+0.293	415, 430	4.0
30.	Adrenalone	−0.480	290	3.6

[a] Values at pH 7.0, 25°, from ref 19–21. [b] Asterisks indicate that "disappearance" or "bleaching" was used to monitor the oxidation of the donor radicals.

anions produced from the acceptors.[22-24] The notation $E^{\circ 1}$ is used to specify the redox potential at pH 7.0, and E_m is used to specify the value at the stated pH at which it is determined. The acceptors used are generally known to produce reversible organic redox systems. For the majority of these acceptors

$$E^{\circ 1} = E^{\circ} - 0.059\text{pH} \qquad \text{(H)}$$

but for dyes and a few other acceptors, the potential values were obtained[20] for the particular pH of the experiment. Equation H does not hold for compounds which undergo ionizations over the pH range examined. For a few (6–7) acceptors, the E_m values were derived from polarographic half-wave potentials. These were obtained *vs.* sce and the $E_m = E_{1/2} + 0.244$ relationship was used.

The potentials of the free radicals examined here are all for the one-electron *oxidation* processes, reaction A. *The redox potentials of these free radicals are presented*, however, as *reduction potentials*, in keeping with the International Convention. Hence the sign in eq G is reversed.

The concentrations of the acceptors were kept relatively low (~2–5 × 10^{-5} *M*) in order (a) not to compete with N_2O fo re_{aq}^- and (b) to observe and determine the formation kinetics of $\cdot A^-$ radicals, and (c) with dyes a maximum 2.5 × 10^{-5}*M* concentration was used to minimize their dimerization in water.

Care was taken to minimize the extent of photolysis of the acceptors by excluding light as much as possible. A synchronized shutter, open for ~8 msec, was used to reduce the photolysis by the monitoring light from the 450-W xenon lamp employed.

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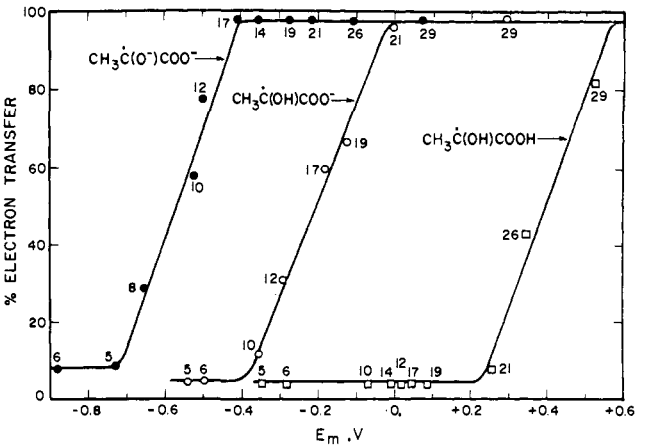

Figure 1. Dependence upon the redox potential of various acceptors A of the efficiency (expressed in percentage) of electron transfer from lactic acid radicals in aqueous solution. Experiments carried out using 5 m*M* lactic acid in the presence of N_2O (1 atm) at pH 3.2 (□), 7.0 (○), and 10.8 (●). Total dose 0.15–1.20 krads/pulse. See Table I for a listing of the acceptors used.

Results

The method used to determine the redox potential of free radicals in aqueous solution was described above in the Experimental Section and is based on studying the extent of formation of $\cdot A^-$ radicals, according to reaction 1. A precise knowledge of the redox potentials $E^{\circ 1}$ of the acceptors A and of the extinction coefficients of $\cdot A^-$ is essential.

The radicals examined below had all been previously studied by pulse radiolysis. Hence their absorption spectra, decay kinetics, and ionization constants had already been established, making it possible to choose carefully and accurately the required experimental conditions.

Lactic and Glycolic Acids. The reaction of OH radicals with lactic acid was shown[25] to produce three forms of free radicals depending on the pH of the solution.

$$\text{OH} + \text{CH}_3\text{CH(OH)COOH} \longrightarrow \text{CH}_3\dot{\text{C}}\text{(OH)COOH} + \text{H}_2\text{O} \quad (7)$$

$$\text{CH}_3\dot{\text{C}}\text{(OH)COOH} \rightleftharpoons \text{CH}_3\dot{\text{C}}\text{(OH)COO}^- + \text{H}^+ \quad \text{p}K_a = 5.3 \quad (8)$$

$$\text{CH}_3\dot{\text{C}}\text{(OH)COO}^- \rightleftharpoons \text{CH}_2\dot{\text{C}}\text{(O}^-\text{)COO}^- + \text{H}^+ \quad \text{p}K_a = 9.8 \quad (9)$$

Based on preliminary results,[9] it was clear that the electron transfer properties of these three radical forms should be different from each other.

The $CH_3\dot{C}(OH)COOH$ radical was studied at pH 3.2 in solutions containing ~5 m*M* lactic acid, 1 atm of N_2O, and 20–50 μ*M* concentrations of acceptors. Essentially no formation of $\cdot A^-$ radicals, and hence no electron transfer, could be observed with menaquinone as an acceptor ($E^{\circ 1} = +0.002$ V). On using acceptors with a higher redox potential, it was possible to reduce them and form $\cdot A^-$; see Figure 1. From the midpoint of this "titration" curve, an $E_m = +0.40$ V at pH 3.2 can be derived. Based on eq G and H this value can be corrected and normalized for pH 7.0 to give $E^{\circ 1} = +0.25$ V.

The $CH_3\dot{C}(OH)COO^-$ and $CH_3\dot{C}(O^-)COO^-$ radicals

(25) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, 73, 4214 (1969).

Table II. Redox Potentials of Some Simple Aliphatic Free Radicals in Water

Substrate	pK_a (radical)	Radical form	pH	E_m, V[a]	$E^{\circ 1}$, V[a,b]
Lactic acid	5.3[c]	$CH_3\dot{C}(OH)COOH$	3.2	+0.40	+0.25
	9.8[c]	$CH_3\dot{C}(OH)COO^-$	7.0	−0.20	−0.12
		$CH_3\dot{C}(O^-)COO^-$	10.8	−0.56	−0.47
Glycolic acid	8.8[c]	$\dot{C}H(OH)COO^-$	7.0	−0.36	−0.28
		$\dot{C}H(O^-)COO^-$	10.0	−0.66	−0.57
Glycolamide	5.5[d]	$\dot{C}H(OH)CONH_2$	3.2	+0.34	+0.19
		$\dot{C}H(O^-)CONH_2$	7.0	−0.26	−0.17
Lactamide	6.5[d]	$CH_3\dot{C}(OH)CONH_2$	3.2	+0.20	0.05
		$CH_3\dot{C}(O^-)CONH_2$	8.5	−0.43	−0.34
Isopropyl alcohol		$(CH_3)_2\dot{C}OH$	1.5	−0.58	−0.83
	12.2[e]	$(CH_3)_2\dot{C}OH$	7.0	−0.90	−0.82
					−1.05[b]
		$(CH_3)_2\dot{C}O^-$	13.0	<−1.40	<−1.31
			13.5	∼−1.95[f]	∼−1.95[f]
Ethyl alcohol	11.6[e]	$CH_3\dot{C}HOH$	7.0	−0.77	−0.69
					−0.93[f]
		$CH_3\dot{C}HO$	13.0	<−1.40	<−1.31
			13.5	∼−1.65[f]	∼−1.65[f]
Methyl alcohol	10.7[e]	$\dot{C}H_2OH$	7.0	−0.73[f]	−0.73[f]
		$\dot{C}H_2O^-$	11.8	<−1.40	<−1.31
			13.5	−1.48[f]	∼1.48[f]
Ribose		$\cdot C_5H_9O_5$	7.0	−0.05	+0.03
			7.0	∼−0.65	∼−0.57
Deoxyribose	9.8[g]	$\cdot C_5H_9O_4$	7.0	−0.19	−0.11
			7.0	−0.65	−0.57
Formate	1.6[h]	$\cdot CO_2^-$	7.0	≪−0.50	≪−0.42
			6.0	−1.09[f]	−1.09[f]
Oxaloacetate	9.2[i]	$\dot{C}(OH)COO^-$	7.0	−0.16	−0.29[l]
		$\lvert$			
		CH_2COO^-			
		$\dot{C}(O^-)COO^-$	10.8	<−0.90	<−1.03[l]
		$\lvert$			
		CH_2COO			
Glycine	6.6[j]	$NH_2\dot{C}HCOO^-$	8.0	−0.87	−0.73
Glycine anhydride	9.6[k]	$NH\dot{C}HCONHCH_2CO$	7.0	∼+0.40	∼+0.49
		$N\dot{C}HCONHCH_2CO$	10.8	−0.48	−0.39

[a] Values to ±0.02 V. [b] Values at pH 7.0 and corrected based on eq G and H. [c] From ref 25. [d] From ref 27. [e] From ref 28. [f] From ref 3. [g] From ref 29. [h] From ref 30. [i] This work. [j] From ref 31 and 32. [k] From ref 33. [l] Derived using eq B in ref 36.

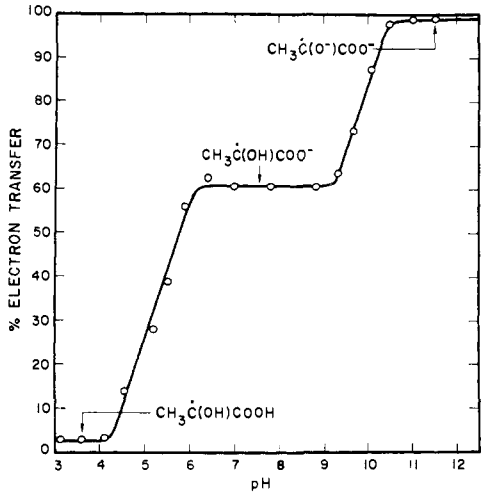

Figure 2. Dependence upon pH of the efficiency of electron transfer from various ionic forms of the lactic acid radicals to anthraquinone-2,6-disulfonate, as the acceptor A. Lactic acid and acceptor 10 mM and 50 μM, respectively, were used in the presence of N_2O (1 atm). Total dose ∼1.2 krads/pulse.

were studied in a similar way at pH 7.0 and 10.8, respectively. Ionization of the carboxyl group considerably increased the reducing properties of this radical; see Figure 1. Ionization of the hydroxyl group increased further the ability of this radical to reduce a whole range of acceptor molecules. Thus, the $E^{\circ 1}$ values for these two radicals were determined to be −0.12 and −0. 47 V, respectively (see Table II).

To confirm the above results and the interpretation given to them, these radicals were titrated against *one* acceptor as a function of pH. The acceptor 9,10-anthraquinone-2,6-disulfonate was chosen based on the results obtained in Figure 1. On varying the pH, two titration curves are observed (see Figure 2) from which one derives pK_a values of 5.2 and 9.8. These values are in excellent agreement with the results previously obtained[25] by following the optical absorbance of the radicals as a function of pH.

This method for titration of free radicals has, in fact, recently been used [8,12] to obtain the ionization constants of free radicals which could not be derived by direct spectrophotometric determination due to the following: (a) the absorption spectrum of the free radical is at an inaccessible wavelength region, and/or (b) the absorption spectra of the acid and base forms of the radicals are too similar and cannot be readily differentiated.

Similar experiments were carried out for glycolic acid, and the results are shown in Figure 3a and Table II. It is interesting to note[26] that the redox potentials of the $\dot{C}H(OH)COO^-$ and $\dot{C}H(O^-)COO^-$ radicals are

(26) It is not clear at present why only ∼70% transfer is observed from the glycolate radicals. OH radical attack to give other radicals cannot be excluded.

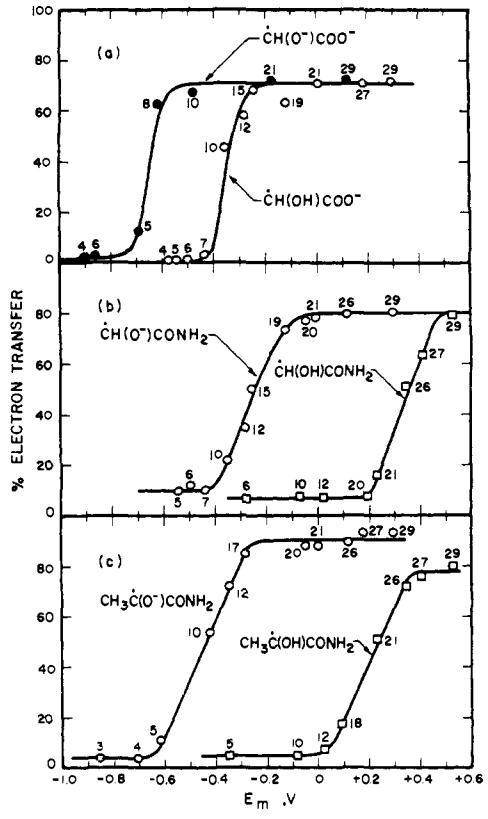

Figure 3. Dependence upon the redox potential of various acceptors A of the efficiency of electron transfer from free radicals produced from (a) glycolic acid at pH 7.0 (○) and 10.0 (●); (b) glycolamide at pH 7.0 (○) and 3.2 (□); (c) lactamide at pH 8.5 (○) and 3.2 (□). Experiments done in 1 atm of N_2O and total dose ~0.15–1.20 krads/pulse. See Table I for a listing of acceptors used.

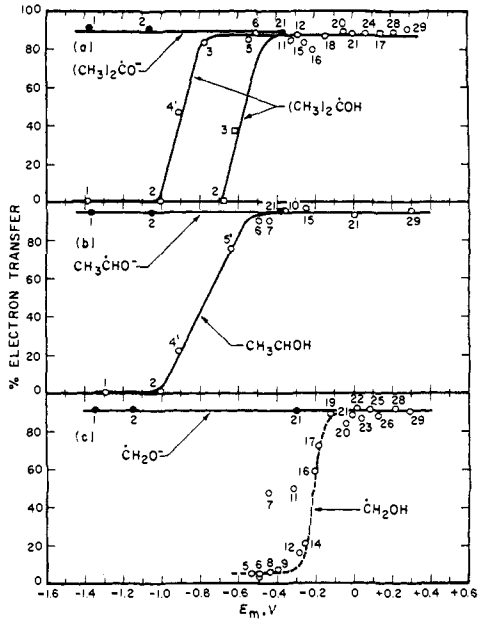

Figure 4. Dependence upon the redox potential of various acceptors A of the efficiency of electron transfer from free radicals produced from (a) isopropyl alcohol at pH 1.5 (□), 7.0 (○), and 13.0 (●); (b) ethyl alcohol at pH 7.0 (○) and 13.0 (●); (c) methyl alcohol at pH 7.0 (○) and 11.8 (●). Experiments done in 1 atm of N_2O, total dose ~0.15–1.20 krads/pulse. See Table I for a listing of acceptors used.

both lower (*i.e.*, have a more negative voltage) than those of the corresponding lactate radicals. The inductive effect of the methyl group apparently makes the lactate radicals weaker reducing agents.

The electron transferred from the lactate and glycolate radicals comes from the Ċ(OH) group and not from the carboxyl group. For the various forms of these radicals, pyruvic acid is produced as a product, *e.g.*

$$CH_3\dot{C}(OH)COO^- + A \longrightarrow CH_3COCOO^- + \cdot A^- + H^+ \quad (10)$$

$$CH_3\dot{C}(OH)COOH + A \longrightarrow CH_3COCOOH + \cdot A^- + H^+ \quad (11)$$

To show that the ionized (or nonionized) carboxyl group is not involved in these electron transfer reactions, the radicals produced from glycolamide and lactamide were studied. These radicals were found to transfer electrons to various acceptors; see Figures 3b and 3c and Table II. Their redox potentials are different from each other and different from the corresponding lactate and glycolate radicals.

Alcohols and Sugars. The reaction of OH radicals with aliphatic alcohols produces free radicals[16,28] which have acid–base properties.[16,28]

$$OH + R_1R_2CHOH \longrightarrow R_1R_2\dot{C}OH + H_2O \quad (12)$$

$$\dot{C}H_2OH \rightleftharpoons \dot{C}H_2O^- + H^+ \qquad pK_a = 10.7 \quad (13)$$

$$CH_3\dot{C}HOH \rightleftharpoons CH_3\dot{C}HO^- + H^+ \qquad pK_a = 11.6 \quad (14)$$

$$(CH_3)_2\dot{C}OH \rightleftharpoons (CH_3)_2\dot{C}O^- + H^+ \qquad pK_a = 12.2 \quad (15)$$

These ketyl radicals have been used as reducing agents for some years. More recently, using a pulse radiolysis combined with polarography technique, the half-wave potentials $E_{1/2}$ of these radicals were obtained.[3] We have used our electron transfer method to determine the $E^{\circ 1}$ of these radicals and compare these values with those observed by Lilie, *et al.*[3] The results obtained are shown in Figure 4 and Table II.

The oxidation potential of the $(CH_3)_2\dot{C}OH$ radical was found to be $E^{\circ 1} = -0.83$ V. The value derived from polarographic studies[3] was $E_{1/2} = -1.30$ V, *i.e.*, $E^{\circ 1} = -1.05$ V. This difference is significant even if one considers the differences in the techniques used and the inherent assumptions and approximations made in each case. This difference could be due, in part, to the irreversible formation of this radical under polarographic experimental conditions (see Discussion). The $CH_3\dot{C}HOH$ radical has a $E^{\circ 1} = -0.69$ V compared to $E^{\circ 1} = -0.93$ V derived polarographically.[3] The determination of the redox potential of the $\cdot CH_2OH$ radical gave irreproducible and scattered results, as shown in Figure 4. The reason for this behavior is not apparent. Polarographically, $E^{\circ 1} = -0.73$ V was found.[3]

The basic form $R_1R_2\dot{C}O^-$ of these ketyl radicals has been known for some time to be a much stronger reducing agent than $R_1R_2\dot{C}OH$, capable of transferring an electron to various acceptors quite efficiently. As expected, their redox potentials are significantly lower. Only an upper limit for the $E^{\circ 1}$ of the $R_1R_2\dot{C}O^-$ radicals was obtained (Figure 4 and Table II) due to the lack of

(27) J. Bell, E. Grunwald, and E. Hayon, to be submitted for publication.

(28) K. D. Asmus, A. Henglein, A. Wigger and G. Beck, *Ber. Bunsenges. Phys. Chem.*, 70, 756 (1966).

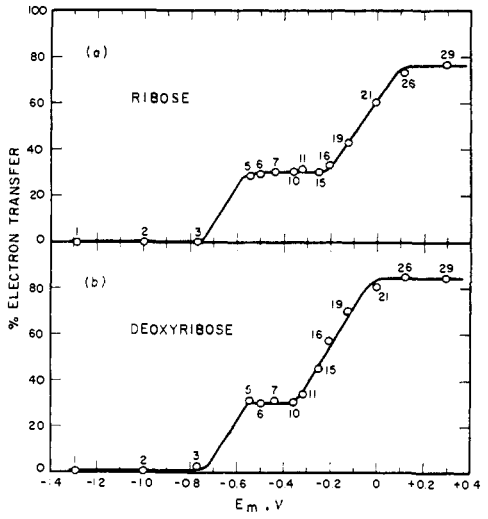

Figure 5. Dependence upon the redox potential of various acceptors A of the efficiency of electron transfer from radicals produced from (a) ribose and (b) deoxyribose, at pH 7.0. Experiments done in 1 atm of N_2O, total dose ~0.15–1.20 krads/pulse. See Table I for a listing of acceptors used.

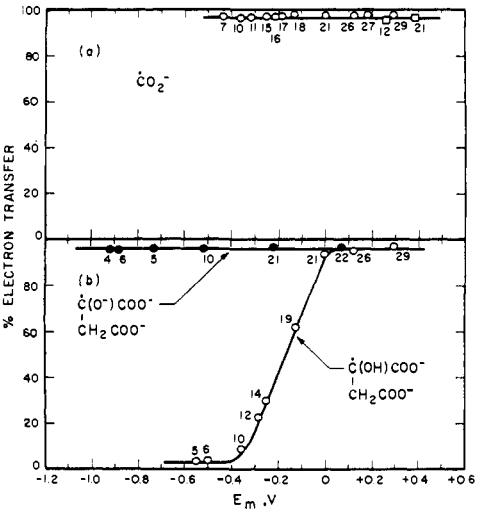

Figure 6. Dependence upon the redox potential of various acceptors A of the efficiency of electron transfer from (a) $\cdot CO_2^-$ at pH 7.0 (○) and $\cdot CO_2H$ radicals at pH 0.5 (□), and (b) radicals produced from the reaction of OH with oxaloacetic acid at pH 7.0 (○) and 10.8 (●). Experiments done in 1 atm of N_2O and total dose ~0.15–1.20 krads/pulse. See Table I for a listing of acceptors used.

appropriate acceptors presently available which can be studied at the relatively high pH $\geq$12. The values obtained polarographically are given in Table II.

Hydroxyl radicals are known to react effectively with the sugars ribose and deoxyribose, abstracting an H atom at more than one CHOH position. The radicals produced are ketyl type radicals and undergo acid–base reactions.[15,29] The ionization constant of the $\cdot C_5H_9O_4$ from deoxyribose has[29] a pK_a = 9.8. At pH 7.0 the dependence, upon the $E^{\circ 1}$ of the acceptors, of the percentage efficiency of electron transfer from these radicals showed two steps on the curve; see Figure 5. This would seem to clearly indicate that the reaction of OH radicals with ribose and deoxyribose at pH 7.0 produces, in each case, at least two radicals, each having a different redox potential (see Table II). Hence one radical is a stronger reducing agent than the other.

It is important to point out that this method, being based on a *quantitative* determination of the efficiency of electron transfer (from 0–100%), provides, in addition to the determination of the redox potential of the radicals, evidence for the formation and amount of different radicals produced in the system under investigation. Thus, for both ribose and deoxyribose ~30% of the OH radicals produce a radical with an $E^{\circ 1}$ of −0.57 and −0.57 V, respectively, and ~50% of the OH produce a radical with a $E^{\circ 1}$ of +0.03 and −0.11 V, respectively. The remaining ~20% of the OH radicals presumably attack these sugars at a third site.

Acids. The $\cdot CO_2^-$ radical has been used frequently as a strong reducing agent.

$$\cdot CO_2^- + A \longrightarrow CO_2 + \cdot A^- \quad (16)$$

It is usually produced *via* reaction 17

$$OH + HCO_2^- \longrightarrow \cdot CO_2^- + H_2O \quad (17)$$

The determination of the redox potential of $\cdot CO_2^-$ was attempted (Figure 6a). Considerable scatter was obtained with acceptors having a $E^{\circ 1} < \sim -0.5$ V. The problem appears to be due to the difficulty of adjusting the experimental conditions such that $k_{16} \gg k(\cdot CO_2^- + \cdot CO_2^-)$. It was hence not possible to obtain a precise value. Polarographically, $E^{\circ 1} = -1.03$ V was obtained.[3] The $\cdot CO_2^-$ can protonate to produce the $\cdot CO_2H$ radical, with a $pK_a \sim 1.6$ (ref 30). The acid form is expected to have a significantly higher (more positive) $E^{\circ 1}$ value.

Oxaloacetic acid is an important intermediate in biochemical pathways. Its role may be as a mediator in electron transfer reactions. The addition of a hydrated electron produces the corresponding ketyl radical

$$e_{aq}^- + {}^-OOCCOCH_2COO^- \longrightarrow {}^-OOC\dot{C}(O^-)CH_2COO^- \quad (18)$$

$${}^-OOC\dot{C}(O^-)CH_2COO^- + H_3O^+ \rightleftharpoons C\dot{O}{}^-OO(OH)CH_2COO^- + H_2O \quad (19)$$

with an ionization constant pK_a = 9.2. At pH 7.0, this radical is present as its acid form and a $E^{\circ 1} = -0.29$ V was obtained; see Figure 6b and Table II. The basic form of this radical is a much stronger reducing agent and a redox potential value $E^{\circ 1} < -1.03$ V was obtained.

Glycine and Glycine Anhydride. Hydroxyl radicals react with the amino acid glycine to abstract an H atom from the carbon atom. The radical produced[31,32] then

$$OH + \overset{+}{N}H_3CH_2COOH \longrightarrow \overset{+}{N}H_3\dot{C}HCOOH + H_2O \quad (20)$$

$$\overset{+}{N}H_3\dot{C}HCOOH \rightleftharpoons NH_2\dot{C}HCOOH + H^+ \quad pK_a = \leq 1.0 \quad (21)$$

$$NH_2\dot{C}HCOOH \rightleftharpoons NH_2\dot{C}HCOO^- + H^+ \quad pK_a = 6.6 \quad (22)$$

undergoes deprotonation in two stages, reactions 21 and 22. At pH 8.0, the $NH_2\dot{C}HCOO^-$ radical is the predominant species. Its electron transfer characteris-

(29) M. Simic and E. Hayon, to be submitted for publication.

(30) G. V. Buxton and R. M. Sellers, *J. Chem. Soc., Faraday Trans. 1*, **69**, 555 (1973).

(31) P. Neta, M. Simic, and E. Hayon, *J. Phys. Chem.*, 74, 1214 (1970); *ibid.*, **76**, 3507 (1972).

(32) H. Paul and H. Fischer, *Helv. Chim. Acta*, 54, 485 (1971).

tics to various acceptors are shown in Figure 7a. From these results, a $E^{\circ\,1} = -0.73$ V was obtained, indicating that this radical is a strong reducing agent. It has already been suggested[7,9] that the electron comes from the amino group (and not the carboxyl group), producing a dehydro derivative which is short lived due to hydrolysis by water.

The peptide radical[33] I has been produced from the reaction of OH radicals with glycine anhydride.

$$\underline{\text{NHCH}_2\text{CONHCH}_2\text{CO}} + \text{OH} \longrightarrow \underset{\text{I}}{\underline{\text{NHCHCONHCH}_2\text{CO}}} + \text{H}_2\text{O} \quad (23)$$

This radical undergoes acid–base reactions

$$\underline{\text{NHCHCONHCH}_2\text{CO}} \rightleftharpoons \underline{\text{NCHCONHCH}_2\text{CO}} + \text{H}^+ \qquad pK_a = 9.6 \quad (24)$$

The acid form of this radical is a poor reducing agent and consequently its redox potential is fairly high, $E^{\circ\,1} \sim +0.49$ V; see Figure 7b and Table II. On the other hand, the basic form of this peptide radical is a relatively strong reducing agent, and a redox potential of $E^{\circ\,1} = -0.39$ V was obtained.

It was conclusively established[7,9,12] that the electron transferred comes from the peptide hydrogen

$$\underline{\text{NHCHCONHCH}_2\text{CO}} + \text{A} \longrightarrow \underline{\text{N=CHCONHCH}_2\text{CO}} + \cdot\text{A}^- + \text{H}^+ \quad (25)$$

$$\underline{\text{NCHCONHCH}_2\text{CO}} + \text{A} \longrightarrow \underline{\text{N=CHCONHCH}_2\text{CO}} + \cdot\text{A}^- \quad (26)$$

since the sarcosine anhydride radical

$$\underline{\text{N(CH}_3\text{)CHCON(CH}_3\text{)CH}_2\text{CO}}$$

did not transfer an electron to any of the acceptors studied.

Discussion

The results presented above would seem to demonstrate the viability of the method described for the determination of the redox potentials of free radicals in aqueous solutions. This method is dependent on the transfer of an electron from a radical donor to a stable ground state acceptor molecule A. A wide range of acceptors have been used in this investigation, ranging from quinones, dyes, aromatic carbonyl compounds, NAD^+, and riboflavin. All these compounds, with $E^{\circ\,1}$ values ranging from -1.30 to $+0.50$ V, respond and fit in well in the experimental "titration" curves obtained from the plot of percentage electron transfer *vs.* E_m of acceptors.

The redox potentials of free radicals vary with pH according to eq H provided the radical does not ionize or protonate over the pH range examined. This is demonstrated for the $(CH_3)_2\dot{C}OH$ radical (see Figure 4a and Table II). At pH 1.5 and 7.0, identical $E^{\circ\,1}$ values were observed.

Considerable care was given to make sure that under the experimental conditions used the donor radicals did *not* disappear by radical–radical reactions but reacted solely with the acceptors. It should be pointed out, however, that with acceptors having much lower $E^{\circ\,1}$ values than those of the radicals, an observed 0% transfer does not necessarily mean that the reaction does not occur. It only means that its rate is relatively very low, $<10^6\ M^{-1}\ \text{sec}^{-1}$, and that it may occur when no other competitive reaction paths are available. The rates of electron transfer reactions as a function of the $E^{\circ\,1}$ value of the acceptors also give[10,3] titration type curves, from which similar $E^{\circ\,1}$ values for the donor radicals can be derived. Generally speaking, k_1 is ~ 3–5×10^8 to $5 \times 10^9\ M^{-1}\ \text{sec}^{-1}$ for acceptors which have a $E_A^{\circ\,1} \geq E^{\circ\,1}_{RH}$.

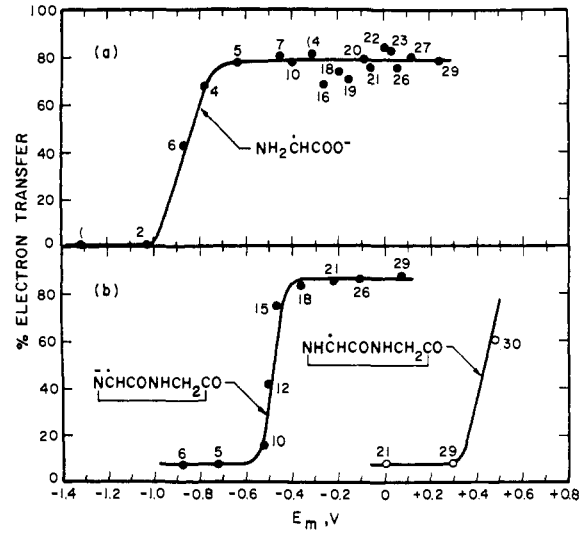

Figure 7. Dependence upon the redox potential of various acceptors A of the efficiency of electron transfer from radicals produced from (a) glycine at pH 8.0 and (b) glycine anhydride at pH 7.0 (○) and 10.8 (●). Experiments done in 1 atm of N_2O and total dose ~0.15–1.20 krads/pulse. See Table I for a listing of acceptors used.

It should be pointed out that the slopes of the "titration" curves (Figures 1–7) vary and the reasons for this are not clear at present. Part of the change in the slopes is probably due to experimental factors, such as an insufficient number of acceptors and scatter of the data. One should also consider the possibility that some of these systems may not be fully reversible, leading to departure from equilibrium conditions, as required by the Nernst equation, and to some errors in the experimentally derived $E^{\circ\,1}$ values. The solvation of the radicals, the formation of short-lived complexes between the radicals and the acceptors, and the nature of the electron transfer mechanism (outer or inner sphere) must be considered and could account for the change in the "slopes" of the titration curves.

The advantages of this method over polarographic or conductometric pulse radiolysis techniques for determining the half-wave potentials of free radicals are: (a) a wide range of radicals and radical anions can be produced dy oxidation of the substrates by OH radicals or by reduction with e_{aq}^-, without the polarographic interference which some substrates demonstrate; (b) the pH, ionic strength of the solution and the charge on the radical do not interfere; (c) the difference in the redox potentials of both the acid and base forms

(33) E. Hayon and M. Simic, *J. Amer. Chem. Soc.*, **94**, 42 (1972).

of the radicals can be directly and accurately determined; (d) there are no surface reactions at the electrodes; (e) quantitative evaluation of different radicals produced (*e.g.*, by attack of OH radicals at more than one site on the molecule) can be obtained; (f) rates of the electron transfer processes can readily be obtained; (g) the ionization constants (pK_a) of free radicals can be determined by this method (this is particularly useful when, due to experimental limitations, the radical cannot be directly observed).

On the other hand, this method has a number of limitations: (a) a very large number of acceptors are needed, and their redox potentials must be well established; (b) the free radical chemistry of the acceptors must be known in order to choose the proper experimental conditions of wavelength, extinction coefficient, pH, and dosimetry; (c) suitable acceptors must be found having very low and very high redox potentials; (d) the acceptors must be stable at the pH of the experiment and must not react thermally with the substrates; (e) in each case, one must establish that $\geq 90\%$ of the radicals can react with A, *i.e.*, that $\leq 10\%$ of the radicals are lost *via* reaction 5.

A knowledge of the redox potential of free radicals is of considerable importance in understanding the mechanism of free radical reactions and the reaction rate constants of electron transfer processes (reaction 1). Indeed, it is now possible based on the $E^{\circ 1}$ values of radicals given in Table II to predict the feasibility and the course of chemical reactions, provided the $E^{\circ 1}$ value of the reactant (acceptor) is known.

For example, published results can now be understood. The $(CH_3)_2\dot{C}OH$ radical ($E^{\circ 1} = -0.82$ V) was found[23] not to transfer an electron to benzophenone ($E^{\circ 1} = -1.0$ V) to form the semiquinone radical $Ph\dot{C}(OH)Ph$ or $Ph\dot{C}(O^-)Ph$. However, $(CH_3)_2\dot{C}O^-$ ($E^{\circ 1} < -1.3$ V) transfers[23] with 100% efficiency and a $k = 1.6 \times 10^9\ M^{-1}\ sec^{-1}$. Similarly, the $CH_3\dot{C}HOH$ radical ($E^{\circ 1} = -0.69$ V) does not transfer to benzophenone but transfers effectively to *N*-ethylmaleimide[6] ($E^{\circ 1} \sim -0.5$ V) and *N*-methylnicotinamide[34] ($E^{\circ 1} = -0.42$ V).

It can be seen from the above results that (a) electron transfer will occur to acceptor molecule having a higher (more positive) potential (*i.e.*, in an upward direction as represented in the table). Many of the reported (see, *e.g.*, ref 35) observations on so-called electron cascading processes can now be rationalized to fit perfectly well on the basis of the redox potentials of the donor and acceptor molecules. (b) Electrons can also, in principle, be transferred from one radical $\cdot R_1H$ to another radical $\cdot R_2H$, provided the second radical has a higher oxidation potential. Thus many radical–radical ($\cdot R_1H + \cdot R_2H$) reactions take place by an electron transfer mechanism, what is normally referred to as disproportionation reactions, to give $R_1 + R_2H_2$ as final products.

In conclusion, it can be stated that in all cases examined above, and with other free radicals,[36,37] the redox potentials of organic free radicals are more negative for the acid form of the free radicals than for the base form of the same radical; see Table II. For example, $E^{\circ 1}$ of $CH_3\dot{C}(OH)COOH > CH_3\dot{C}(OH)COO^- > CH_3\dot{C}(O^-)COO^-$, namely $+0.25$, -0.12, and -0.47 V, respectively. This means that the reducing power of free radicals is greatest for the ionized forms of these radicals.

(34) U. Bruhlman and E. Hayon, *J. Amer. Chem. Soc.*, submitted for publication.
(35) G. E. Adams and R. L. Wilson, *J. Chem. Soc., Faraday Trans. 1*, **69**, 719 (1973).
(36) P. S. Rao and E. Hayon, *J. Amer. Chem. Soc.*, **96**, 1295 (1974).
(37) P. S. Rao and E. Hayon, *Biochem. Biophys. Acta*, submitted for publication.