[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE]

Oxidation–Reduction Potentials and Ionization Constants of the Reversible Series: Hexahydroxybenzene–Tetrahydroxyquinone–Rhodizonic Acid

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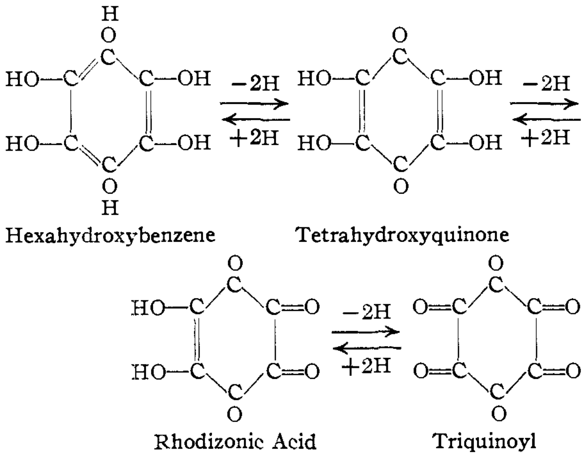
The oxidation–reduction potentials of the interesting reversible series



which involves six equivalents on the same organic ring structure, has been studied as part of an investigation of the potentials of type systems and the effect of structure on potentials and also as part of an investigation of the pathway of oxidation of inositol (cyclic $(CHOH)_6$).

The first two divalent change steps of this series involving a total of four oxidation–reduction equivalents could be measured over a wide range of *p*H and it was found that in one zone a significant amount of the tetravalent change equilibrium from hexahydroxylbenzene to rhodizonic acid also occurs. The third step of two equivalents to form triquinoyl could not be successfully measured because of the instability of this substance. Further study of systems involving six equivalents on the same organic structure is being made with the corresponding mono-, di- and tri-phenazines.

The oxidation of inositol (a substance found widely distributed in nature as its phosphate esters and an essential substance for the life processes of some organisms) by certain bacteria passes partially through this series of compounds producing a red precipitate of calcium rhodizonate.[1]

The oxidation reduction potential apparatus and procedure for conducting titrations in the absence of oxygen previously described were employed.[2] Titrations were made at 30° of buffered solutions of potassium rhodizonate with buffered sodium hydrosulfite ($Na_2S_2O_4$) at *p*H 6.74 and higher and also of reduced solutions, prepared by means of colloidal palladium and hydrogen with subsequent reoxidation with ferricyanide, at all *p*H recorded. Preparation of the potassium rhodizonate, a characteristic titration, and some of the possible errors involved in the titration have been described in a publication on this salt.[3] The compositions of the 0.1 *M* buffers used were: *p*H 3.51 through 5.80 citrate; 6.02 through 7.46 phosphate; 7.77 through 8.87 veronal; and 9.36 through 9.88 carbonate. Potentials are referred to the normal hydrogen electrode.

Titration curves of E_h plotted against equivalents of oxidant or reductant are shown in Fig. 1. Graphical and mathematical analysis of the individual sections of the curves, where two distinct separated steps occur, were made on the basis of the equation

$$E_h = E_0' - \frac{RT}{nF}\log_e\frac{(\text{red.})}{(\text{ox.})}.$$

In the alkaline region of *p*H 7.46 and beyond, the difference in volts between the $^1/_2$ and 1 equivalent points or the 1 and $1^1/_2$ (or the $2^1/_2$ and 3 or the 3 and $3^1/_2$) is 0.014 to 0.016 volt indicating two divalent steps with little evidence of semiquinone formation.[4,5]

On decreasing the *p*H, the steps gradually merge forming an apparently single curve. The least slope attained, 0.016 volt, for the change from 1 to 2 (or from 2 to 3) equivalents, occurs at *p*H 5.80. Here the curve is tending toward a single tetravalent type curve where *n* in the electrode equation is 4. On further decrease of *p*H the curve again separates into two steps. The pure tetravalent type curve with a slope of 0.007 volt is not attained because of the particular relationship of the ionization constants of the components.

Several systems involving two di-valent type curves have been reported in the literature,[6,7] but sufficient overlapping or merging of the curves to reveal a four-valent character of the systems was not apparent.

The graphical construction and mathematical formulation of the theoretical curves of E_h against equivalents can be readily accomplished. The family of curves (E_h plotted against equivalents of oxidant or of reductant) for the transformation of two di-valent curves into a single four-valent curve can be prepared from the

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(1) A. J. Kluyver, T. Hof and A. G. J. Boezaardt, *Enzymologia*, **7**, 257 (1939).

(2) P. W. Preisler and L. H. Hempelmann, THIS JOURNAL, **58**, 2305 (1936).

(3) P. W. Preisler and L. Berger, *ibid.*, **64**, 67 (1942).

(4) B. Elema, *Rec. trav. chim.*, **50**, 807, 1004 (1931); *J. Biol. Chem.*, **100**, 149 (1936).

(5) L. Michaelis, *Chem. Rev.*, **16**, 243 (1935); L. Michaelis and M. P. Schubert, *ibid.*, **22**, 437 (1938).

(6) R. K. Cannon, *Biochem. J.*, **20**, 927 (1926).

(7) L. F. Fieser and M. A. Peters, THIS JOURNAL, **53**, 798 (1931).

graph of the semiquinone family (two uni-valent curves transforming into a single di-valent curve[4,5]) simply by renumbering the units of the "equivalent" axis so that they are twice as high numerically.

The values for the characteristic points on the curves can be calculated with modified equations derived by inserting the proper value for n, the number of electrons involved in the changes, in the equations[4] applying to semiquinone formation. The final modified equations become

$$K = (153.26\,S - 2)^2, \text{ and}$$
$$E_2 - E_1 = 0.03006 \log_{10} K$$

where K is the constant for the formation of tetrahydroxyquinone from rhodizonic acid and hexahydroxybenzene, E_1 and E_2 are the E_0' potentials of the respective di-valent steps, and S is the voltage difference between the center of the curve at 2 equivalents and the point of intersection with the E_h axis of the tangent to the curve at the center.

The equilibria existing in the *p*H region around 5.8 may also be visually demonstrated by adding solid tetrahydroxyquinone to deoxygenated buffer. The deep yellow color of rhodizonate ions immediately appears indicating the dismutation of part of the tetrahydroxyquinone into rhodizonate ion (and hexahydroxybenzene). When added to deoxygenated buffer at *p*H 10, the solution acquires the pale purple of tetrahydroxyquinone ions.

The principal points of the titration curve are given in Table I, with the calculated E_0' of the separate steps. E_1 is the E_0' of the hexahydroxybenzene–tetrahydroxyquinone system, E_2 is the E_0' of the tetrahydroxyquinone–rhodizonic acid system, E_m the E_0' of the hexahydroxybenzene–rhodizonic acid system (also the center of the curve at 2 equivalents), $E_{1/4}$ and $E_{3/4}$ the voltage at 1 and 3 equivalents, respectively, and K and S as defined above.

TABLE I

	Observed values			Calculated values			
*p*H	$E_{1/4}$	E_m	$E_{3/4}$	S	$E_2 - E_1$	E_2	E_1
3.51	0.201	0.169	0.138	0.077	0.059	0.199	0.139
3.84	.177	.147	.117	.071	.057	.175	.118
4.08	.158	.129	.100	.067	.055	.152	.107
4.54	.118	.097	.075	.044	.041	.118	.077
4.98	.075	.058	.041	.035	.032	.074	.042
5.22	.054	.037	.021	.032	.027	.050	.023
5.48	.028	.012	−.003	.031	.026	.025	−.001
5.80	.000	−.016	−.032	.032	.027	−.002	−.029
6.02	−.013	−.032	−.050	.036	.033	−.015	−.048
6.39	−.045	−.063	−.081	.036	.033	−.046	−.079
6.74	−.073	−.096	−.118	.044	.041	−.075	−.116
7.10	−.098	−.128	−.158	.065	.054	−.101	−.155
7.46	−.127	−.165	−.203	.135	.076	−.127	−.203
7.77	−.143	−.187	−.235	.196	.087	−.144	−.231
7.96	−.156		−.257			−.156	−.257
8.12	−.163		−.267			−.163	−.267
8.35	−.180		−.303			−.180	−.303
8.76	−.204		−.350			−.204	−.350
9.36	−.246		−.417			−.246	−.417
9.62	−.257		−.437			−.257	−.437
9.88	−.273		−.460			−.273	−.460

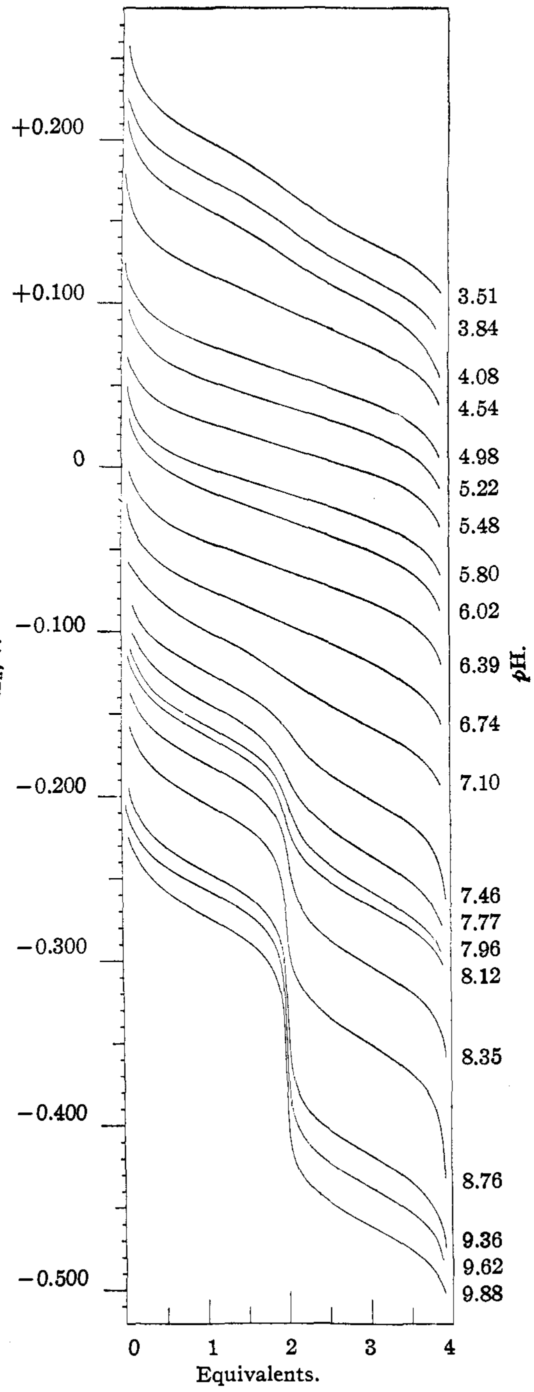

Fig. 1.

The relationship between the E_0' of the system and *p*H and the ionization constants is presented in Fig. 2. The standard slopes have been drawn through the points and the ionization constants of the constituents have been located by the intersections. Each ionization of tetrahydroxyquinone causes a change in slope (ΔE_h divided by ΔpH) of +0.030 volt in each di-valent change curve when it is acting as the oxidant (tetrahy-

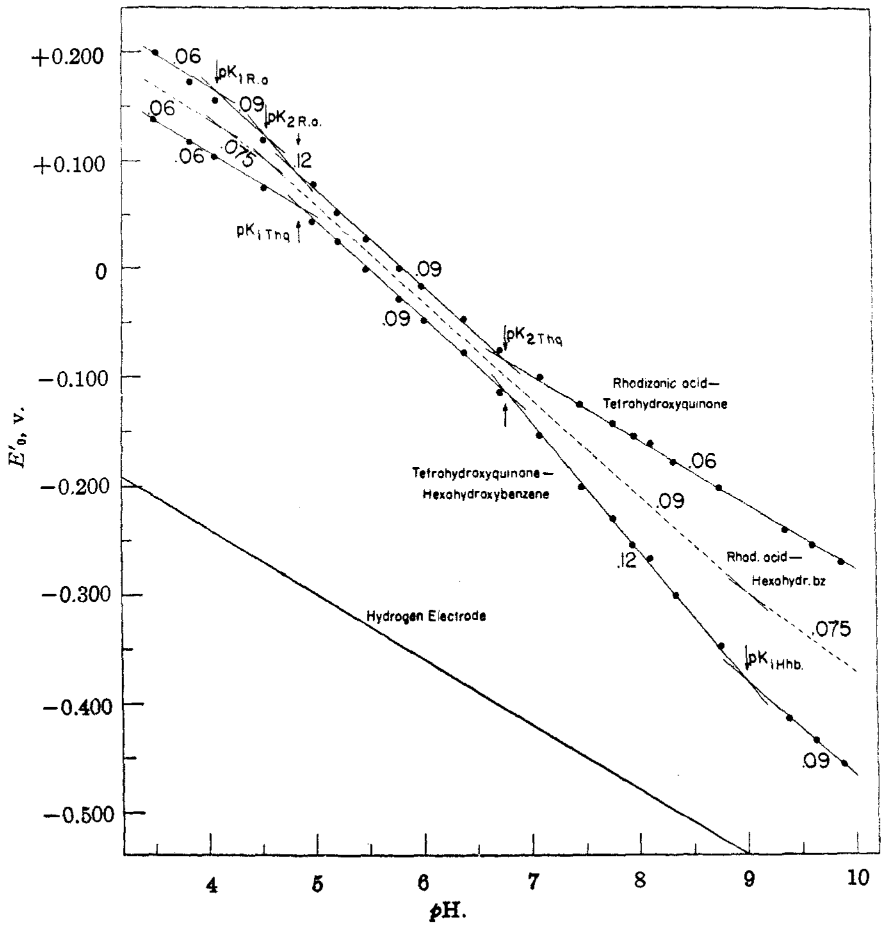

Fig. 2.

droxybenzene–hexahydroxybenzene) and a -0.030 change when it is the reductant (rhodizonic acid–tetrahydroxyquinone); rhodizonic acid causes a $+0.030$ change for each ionization since it is an oxidant; hexahydroxybenzene causes a -0.030 change for each ionization since it is a reductant. Tetrahydroxyquinone does not affect the slope of the tetra-valent change curve because it is not a component of this system, while each of the components of the tetra-valent curve (dotted line) causes a 0.015 volt change.

Two ionizations of rhodizonic acid in the *p*H range between 3.5 and 5.0 were indicated by the results of the titration of 1.23 g. of potassium rhodizonate (0.005 mole) suspended in 20 cc. of water, where 10.5 cc. of *N* hydrochloric acid (0.01 mole) was required for complete solution and removal of essentially all of the deep yellow color of the rhodizonate ion.

The ionization constants found graphically are: for rhodizonic acid pK_1 4.1 and pK_2 4.5; for tetrahydroxyquinone, pK_1 4.8 and pK_2 6.8; and for hexahydroxybenzene pK_1 9.0.

The E_0 of the system estimated by extrapolation of the respective E'_0–*p*H curves to *p*H 0 are: rhodizonic acid – tetrahydroxyquinone, $+0.410$; tetrahydroxyquinone–hexahydroxybenzene, $+0.350$; rhodizonic acid–hexahydroxybenzene, $+0.380$ volt.

The effect of substitution of hydroxyl groups for hydrogen in the quinone structure can be estimated, but only for the most reducing step, tetrahydroxyquinone–hexahydroxybenzene, because of the complexity of the equilibria for the other steps which may involve both *o*- and *p*-quinones. The E_0 of *p*-quinone–hydroquinone[8] is $+0.695$ volt. The difference, $+0.345$, between the value for *p*-quinone–hydroquinone and tetrahydroxyquinone–hexahydroxybenzene is a measure of the effect of the four hydroxyl groups or about -0.088 volt for each hydroxyl. This is of the order of the effect of a hydroxyl group for other hydroxyquinones.[9]

Some deductions of theoretical interest may be made from the foregoing data concerning the preparation of the components and the possible catalytic effects of these systems on oxidation–reduction reactions generally.

The data explain the exclusive precipitation of di-potassium rhodizonate from acetate buffers, in

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(8) E. Billmann, *Bull. soc. chim.*, **61**, 217 (1927).

(9) J. B. Conant and L. F. Fieser, This Journal, **46**, 1858 (1924).

contrast to the precipitation of the di-potassium salt of tetrahydroxyquinone from carbonate buffers, when the nitric acid oxidation products of inositol are oxidized by a stream of air in such solutions.[3] The usual pathway of oxidation of hexahydroxybenzene to rhodizonic acid is stepwise through tetrahydroxyquinone, but in acetate buffers rhodizonic acid is formed also by the dismutation of tetrahydroxyquinone into hexahydroxybenzene and rhodizonate. In carbonate buffers, the di-potassium salt of tetrahydroxyquinone does not dismute into rhodizonate and hexahydroxybenzene but precipitates out as formed, thus preventing further oxidation to rhodizonate. The ionization constants of the two substances are such that precipitation of the di-potassium rhodizonate would be favored in acetate buffers.

The potential of these systems in the normal *p*H range of living cells is such that when present they *might* take part in the oxidation–reduction processes as catalysts offering more rapid di-valent pathways or acting as mediators between tetravalent and di-valent steps according to the "equivalence change principle."[10]

Summary

The oxidation–reduction potentials of the several equilibria involved in the reversible systems composed of hexahydroxybenzene, tetrahydroxyquinone and rhodizonic acid have been measured at various *p*H. From these the E_0 of the systems and the pK of the components have been estimated. Equations are presented which apply to this complex relationship involving one tetravalent system and two di-valent systems in equilibrium.

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[10] P. A. Shaffer, *Cold Spring Harbor Symposia on Quant. Biol.*, **7**, 50 (1939); *J. Phys. Chem.*, **40**, 1021 (1936); THIS JOURNAL, **55**, 2169 (1933)..

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CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CARNEGIE INSTITUTE OF TECHNOLOGY]

The Heat of Combustion of Tungsten Carbide, WC[1]

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The heat of combustion of tungsten carbide, WC, has not been reported in the literature, and this paper describes measurements of this thermal value, as carried out in a precision bomb calorimeter which has been described previously.[3]

The heat of formation of WC has been calculated from these results, but this calculation is subject to the uncertainties in the reported values for the heat of formation of tungstic oxide. Further measurements, in these laboratories, on the heat of combustion of tungsten are contemplated in the near future.

Experimental

Material.—The tungsten carbide used in these measurements was supplied by the McKenna Metals Company. It was reported to have been prepared by dissolving tungsten in molten aluminum and heating to about 2000° in a graphite crucible.[4] The aluminum carbide formed was dissolved from the solidified mass with nitric acid, which does not attack WC. This carbide remains as a shiny macro-crystalline material. Spectroscopic analysis showed it to contain an appreciable amount of nickel, along with several hundreths per cent of molybdenum and columbium, and with lesser amounts of titanium, silicon, calcium, iron, aluminum, magnesium and copper decreasing in this order to less than 0.001%. These results are only roughly quantitative since no adequate standard of this material was available for comparison. Purification of the carbide was effected by passing dry chlorine over it as the temperature was raised slowly to 630°. It has been shown[5] that WC is not affected by such treatment, while any lower carbides and tungsten metal are volatilized. An appreciable amount of volatilizate was obtained by this treatment, and analysis showed it to contain nickel chloride corresponding to 0.17 per cent. of nickel in the original carbide. The residual material was thoroughly washed with ammonia, dilute acid, water, alcohol and ether and then dried. The appearance of the carbide was unchanged and chemical and spectroscopic analysis gave the following results

Chemical analysis	Spectroscopic
Cb, trace	Less than 0.05%
Ni, trace	Less than 0.05%
Fe, trace	Trace
Mo, 0.04%	Less than 0.05%

Combustion analysis for carbon gave 6.128 ± 0.006 per cent., compared to the theoretical value of 6.130 per cent.

The Calorimeter.—In this study the precision bomb calorimeter described by Snyder and Seltz[6] was used. The resistance thermometer was recalibrated and found to have changed inappreciably. The procedure and precautions followed those previously described except that in these measurements it was not necessary to use the large protecting zircon crucible required for the combustion of aluminum. Small fused silica dishes satisfactorily withstood the combustion and the fused tungstic oxide showed no slaging action on the silica.

Calibration of the Calorimeter.—To obtain the heat capacity of the calorimeter benzoic acid was used as a calibrating substance. Standard sample No. 39f was obtained from the Bureau of Standards. Its heat of combustion was reported by R. S. Jessup[7] to be Q (under standard conditions) = 26,428.4 ± 2.6 international

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[1] Abstracted from a thesis presented by L. D. McGraw to the Committee on Graduate Instruction of the Carnegie Institute of Technology, in partial fulfillment of the requirements for the degree degree of Doctor of Science. February, 1946.

[2] Present address: Dept. of Chem., Webster College, Webster Groves, Missouri.

[3] Snyder and Seltz, THIS JOURNAL, **67**, 683 (1945).

[4] R. Mitsche, *Berg huttenmann. Jahrb. montan. Hochschule Leoben*, **87** 135–187 (1939).

[5] Iitaka and Aoki, *Bull. Chem. Soc. Japan*, **7**, 108 (1932); *C. A.*, **26**, 3743 (1932).

[6] Snyder and Seltz, THIS JOURNAL, **67**, 683 (1945).

[7] Jessup, *J. Research Natl. Bur. Standards*, **29**, 247 (1942).