ing to its true diffusion coefficient, $D = RTu/n$. Thus excess of a strong acid or of a strong base could form a perfect buffer, say, for sodium ion diffusing through it, with a rate corresponding to $D = 1.15$.

Since in these cases the movement of the buffer may be comparable to the movement of the particle or ion being studied, an optical method of examination can give erroneous results.

In all of the foregoing discussions such relatively less important influences as activity coefficient and collision effects are omitted; also the influence of ion pairs or ionic complexes[11] is left out of account. The foregoing factors do not affect results of electrolytic migration because in all such measurements the middle portion of the system is free from any concentration gradients. Likewise it does not affect measurements of hydration of ions by movement of a non-electrolytic reference substance, except in so far as collision effects may account for some of the minute movements observed.[12]

(11) McBain and Van Rysselberghe, THIS JOURNAL, **52**, 2336 (1930).

Summary

Diffusion data are supplied for sodium chloride, iodide, thiocyanate, bicarbonate, acid tartrate, tartrate, sulfate and citrate, lithium chloride and tartaric acid.

Developing the classical relations of Nernst and Arrhenius, equations are given for calculating diffusion of ions with, through or against other ions. Effects due to mere concentration gradient are explicitly kept separate from the electrical factor produced by diffusion potential. Only when the latter is zero do ions move at their true intrinsic velocity. In other mixtures fast ions are accelerated and slow ions are retarded and may be almost completely stopped. The fastest diffusion is that of hydrogen ion which may thus be pushed up to 40% above its previously supposed limit of $D = 8$ sq. cm./day.

(12) Taylor and Sawyer, *J. Chem. Soc.*, 2095 (1929).

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[CONTRIBUTION FROM THE EVANS MEMORIAL, MASSACHUSETTS MEMORIAL HOSPITALS, AND BOSTON UNIVERSITY SCHOOL OF MEDICINE]

Amperometric Measurement of the Potency of Catalase

BY BURNHAM S. WALKER

The purpose of the present study was to devise a suitable electrical method for the measurement of catalase, which might offer advantages in precision and convenience over titration methods. Such a method has been reported for the measurement of peroxidase,[1] in which the amount of hydroquinone oxidized to quinone is determined by a measurement of electromotive force. Catalase has been shown to have an effect upon the hydrogen peroxide component of the oxygen wave obtained by the polarograph.[2] We were unable, with the equipment available to us, to make a quantitative application of that particular effect. It was found to be a relatively simple matter to follow the course of the decomposition of hydrogen peroxide by catalase, using a dropping mercury electrode.

Experimental

The electrical circuit used was that of Petering and Daniels[3] using as a source of measured applied e. m. f. a Leeds and Northrup type K potentiometer. A box type of galvanometer was used, equipped with a shunt of nichrome wire. The length of the shunt wire was empirically selected to give full-scale deflection with a measured current of 0.2 milliampere. A fixed resistance of 2000 ohms was introduced into the circuit to aid in the suppression of maxima.[4] The dropping mercury electrode was constructed of capillary tubing obtained from broken clinical thermometers. The mercury was of "C. P." grade, and was cleaned for re-use with a current of air. No specially designed electrode vessel was used. The majority of experiments were carried out in an ordinary 50-cc. beaker. The amount of substrate solution was 25 cc., to which was added during the experiment a freshly prepared dilute solution of crystalline catalase in 0.1 cc. volume. The dropping mercury electrode was made the cathode, the anode being either a quiet pool of mercury at the bottom of the beaker, or a saturated calomel electrode connected by a potassium chloride–agar bridge.

In contrast to most measurements at the dropping mercury cathode, all determinations were carried out in solutions kept saturated with oxygen at atmospheric pressure. This was considered necessary since the decomposition of hydrogen peroxide by catalase yields oxygen, and the final measurements must be made under conditions of saturation with oxygen. All solutions were $M/30$ in phosphate buffer (Sørensen) at pH 7.

(1) Sitharaman and Rengachari, *J. Indian Chem. Soc.*, **14**, 278 (1937).

(2) Brdička and Tropp, *Biochem. Z.*, **289**, 301 (1937).

(3) Petering and Daniels, THIS JOURNAL, **60**, 2796 (1938).

(4) Lingane, *ibid.*, **62**, 1665 (1940).

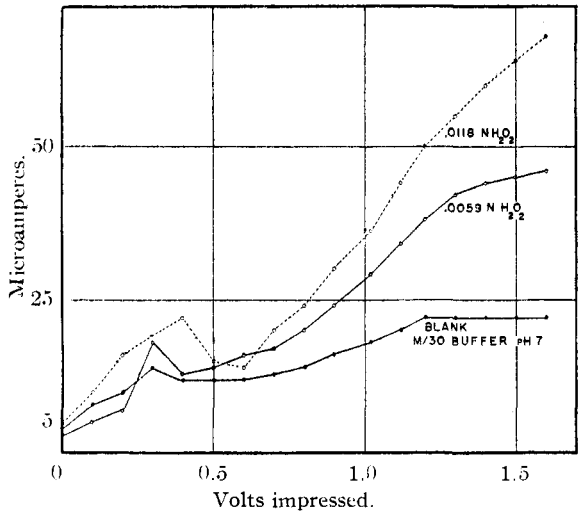

Fig. 1.

Figure 1 shows a current–voltage curve for oxygenated buffer solution, and for the same solution with hydrogen peroxide present in 0.0059 and 0.0118 N concentration. One drop of methyl red (saturated solution in $M/30$ buffer) was added per 50 cc. of solution to aid in the control of maxima. This did not eliminate the primary oxygen maxima, which appear on the graph at 0.3 to 0.4 volt applied. This does not constitute a source of error, since measurements were not made in this range. With the potentiometer used, 1.6 volts was the maximal applied e. m. f.; at 1.5 volts, the applied e. m. f. selected for readings, the relationship between the current and the concentration of hydrogen peroxide, while not linear, was sufficiently constant (with a given electrode) to permit measurements. An explanation for the deviation from linearity appears when the curves for peroxide-containing solutions (Fig. 1) are examined. The curve is still rising at 1.6 volts. It is evident that at 1.5 volts the current measured is not a true limiting current. A further reason for non-linearity is the omission of correction for the iR drop in the circuit.

The actual procedure for determination of reaction constants is as follows: with the electrode in position and with mercury dropping, prepare an approximately 0.02 N solution of hydrogen peroxide by diluting 1.2 cc. of 30% hydrogen peroxide to one liter with water. Prepare three reference solutions by adding to one volume of $M/15$ buffer (pH 7): (a) one volume of water, (b) one-half volume of water and one-half volume of hydrogen peroxide solution, (c) one volume of hydrogen peroxide solution. Add one drop of methyl red solution for each 50 cc. of each of these solutions. The potentiometer being set to apply a voltage of 1.5, the current passing through each of these solutions is measured. These measurements must be made for each day's work, and every time a new electrode or a freshly cleaned electrode is used. Before taking readings, each solution is saturated with oxygen at atmospheric pressure by bubbling oxygen through for five minutes at the rate of two liters per minute.

It will be noted that solution (b) has half the concentration of solution (c) in hydrogen peroxide. Hence its current will represent the current which passes when half the hydrogen peroxide in solution (c) is decomposed. To a measured portion (25 cc.) of solution (c) add the measured solution of catalase to be tested (usually 0.1 cc.) and start a stop watch. Place the solution in the electrode vessel, and make current measurements at 1.5 volts applied e. m. f. until the value of the current falls to the half-value previously determined with solution (b). Stop the watch. To prevent what appears to be supersaturation with oxygen and consequent high current values, the passage of oxygen should be continued for intervals between readings. Oxygen should not be passing at the actual time of measurement, since stirring causes variable readings. On stopping the oxygen flow, the galvanometer steadies very quickly, showing only the fluctuations corresponding to the fall of the mercury drops. The maxima of these fluctuations were taken as the significant readings; it would be more in accordance with sound polarographic practice, although also more laborious, to take the mid-point of the fluctuations.

Under these experimental conditions the reaction constant can be calculated

$$K = \frac{1}{t} \log 2$$

using Briggsian logarithms in accordance with what seems to be the long-accepted custom.[5]

Experimental Results.—Values for K obtained by this method are compared in Table I with values simultaneously determined on the same solutions and for the same time using the titration method of Stern.[6] These measurements were made at room temperature.

TABLE I

SIMULTANEOUSLY MEASURED VELOCITY CONSTANTS OF THE DECOMPOSITION OF HYDROGEN PEROXIDE IN THE PRESENCE OF VARYING AMOUNTS OF CATALASE, MEASUREMENTS AT ROOM TEMPERATURE

K by titration	K by dropping mercury electrode
0.031	0.032
.047	.045
.048	.058
.078	.088
.120	.126
.147	.150

Kat. f. was originally measured at 0°, and this condition has become standardized by usage. Attempts to measure K at 0° by the dropping mercury electrode were unsuccessful; the drop in current lags considerably behind the drop in hydrogen peroxide concentration as measured by titration. This discrepancy may possibly be the result of the persistence at the lower temperature of significant concentrations of an intermediate enzyme-substrate compound, *e. g.*, "re-

(5) Euler, (*Hofmeisters*) *Beiträge zur chemischen Physiologie und Pathologie*, **7**, 1 (1906).

(6) Stern, *Z. physiol. Chem.*, **204**, 259 (1932).

duced catalase."[7] The temperature coefficient of the "over-all" reaction between catalase and hydrogen peroxide has been reported[8] as Q_{10} = 1.4 (0–20°). I have been able to confirm this value in experiments conducted by the titration method.

If one wishes, therefore, to express results in terms of *Kat. f.*, the measurement with the dropping mercury electrode should be made at a temperature in the neighborhood of 20°, and the value of K corrected to 0° by the use of this known value of Q_{10}. The *Kat. f.* of a preparation of crystalline catalase from beef liver[9] was 34,800 by the electrical method, corrected to 0°, and 36,000 by the titration method at 0°. The *Kat. f.* of crystalline catalase is now known to be variable in different preparations.[10] The value obtained is within the limits established by Sumner, Dounce and Frampton.

(7) Keilin and Hartree, *Proc. Roy. Soc.* (London), **B124**, 397 (1938).
(8) Stern, *J. Biol. Chem.*, **114**, 473 (1936).
(9) Sumner and Dounce, *ibid.*, **127**, 439 (1939).
(10) Sumner, Dounce and Frampton, *ibid.*, **136**, 343 (1940).

Other units of catalase potency involving a measurement of the reaction velocity constant[11] may be determined directly or by the use of a temperature correction according to the definition of the unit.

This work was done with the technical assistance of Miss Mary McManus.

Summary

A method is described for the measurement of the velocity of the decomposition of hydrogen peroxide by catalase, utilizing the dropping mercury electrode, without a polarograph or other automatic recording device. The method is applicable at ordinary room temperatures, giving good agreement with titration measurements. At temperatures around 0° it yields consistently lower velocity measurements than those obtained by titration, suggesting the presence at lower temperatures of a measurable concentration of an intermediate enzyme-substrate compound.

(11) Williams, *J. Gen. Physiol.*, **11**, 309 (1927).

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF TORONTO]

The Action of Diazomethane on Lactones and on Lignins

BY E. Y. SPENCER AND GEORGE F. WRIGHT

The reaction of diazomethane with acidic hydroxyl groups has been utilized by some workers as a means of evaluating the amount of such hydroxyl groups in lignins.[1] Others have arbitrarily assumed that isolated lignin was a homogeneous substance having one acidic hydroxyl group per recurring unit in the "molecule." On the basis of diazomethane reaction they have assigned a unit molecular weight to the substance.[2] Such an assumption would seem to be unwarranted from several points of view. First, evidence has been accumulating to indicate that lignin in wood is bound through its phenolic hydroxyl groups. These linkages appear in the extracted materials as phenolic esters of the organic acid (acetic or formic) used as extractant as well as phenol glycosides or phenol glycuronides and their acetates. Such linkages become apparent when the acetic or formic acid-extracted lignin is subjected to either alkaline or acid hydrolysis.[3] These hydrolyses liberate carbohydrate (part of which has been identified as xylose), the organic acid used in the extraction process, and an isolated lignin having a much higher phenolic hydroxyl content, estimated by reaction with diazomethane, than was found in the original extracted lignin. This phenolic hydroxyl content is likewise greater than that found in isolated lignins obtained by more drastic extraction methods involving use of mineral acid or alkali at elevated temperatures. It would indeed appear from the analytical data[3] that these phenolic linkages include most of the hydroxylic groups in the non-saccharide portion of the lignin complex.[4] In

(1) Fuchs and Horn, *Ber.*, **62**, 1691 (1929); Freudenberg and Sohns, *ibid.*, **66**, 262 (1933).
(2) Brauns and Hibbert, THIS JOURNAL, **55**, 4720 (1933); Brauns, *ibid.*, **61**, 2120 (1939).
(3) (a) Bell, Wright and Hibbert, unpublished results completed at McGill University in August, 1937. (b) Lieff, Wright and Hibbert, THIS JOURNAL, **61**, 1477 (1939).
(4) In this Laboratory lignin is regarded, according to the botanical definition, as the non-cellulosic incrusting material in wood xylem. The relationship of this lignin to the materials isolated by various methods and designated by prefix such as Klason lignin, methanol lignin, etc., depends entirely on the method of extraction. Attempts to extract lignin free from detectable carbohydrate in no way demonstrate that the incrusting substance in wood is not partly saccharide in nature. The fact that acetic acid birch lignin contains combined carbohydrate when it is isolated is considered here as evidence that lignin, the cementing material, contains carbohydrate in the wood