tilled until the volume of the residue was about 50 cc. This residual solution was cooled and the ketimine filtered and washed with cold ethanol. Recrystallization from absolute ethanol gave 12.8 g. of colorless crystals which melted at 312–313° (Maquenne block) and possessed a specific rotation of −137.6° in ethanol (*c*, 1). These constants checked with those previously obtained.[1b]

2-(N-Methylamino)-*d*-camphane-10-sulfonic Acid (XI).—2-(N-Methylimino)-*d*-camphane-10-sulfonic acid (18.8 g.) was dissolved in 150 cc. of absolute ethanol and catalytically reduced with hydrogen and platinum-oxide platinum black. It was necessary to use two successive portions of 0.5 g. of catalyst in order to secure complete reduction, which required about twenty-four hours. The catalyst was removed by filtration and the filtrate concentrated. The crude product was fractionated by a series of systematic crystallizations from 95% ethanol. The two diastereoisomers were isolated as colorless crystals.

α-Form.—This form had a decomposition point of 320° and a specific rotation in ethanol (*c*, 1) of −98.6°. *Anal.* Calcd. for $C_{11}H_{21}O_3NS$: C, 53.41; H, 8.56; N, 5.66. Found: C, 53.51; H, 8.54; N, 5.54.

β-Form.—This isomer decomposed at 338–343° and had a specific rotation in ethanol (*c*, 1) of +38.8°. *Anal.* Calcd. for $C_{11}H_{21}O_3NS$: C, 53.41: H, 8.56; N, 5.66. Found: C, 53.52; H, 8.49; N, 5.65.

The α-form was identical with the compound obtained by hydrolysis of the N-methyl sultam (VII).

Conversion of the α-Form of 2-(N-methylamino)-*d*-camphane-10-sulfonic Acid to the Sultam (VII).—One-tenth of a gram of the α-form of 2-(N-methylamino)-*d*-camphane-10-sulfonic acid was heated with 3 cc. of acetic anhydride for eighteen hours. The mixture was then poured into 5 cc. of water and evaporated to dryness. Three cubic centimeters of acetic anhydride was added and the mixture again heated to the boiling point. The mixture was cooled and then 3 cc. of water was added. This mixture was then evaporated to dryness. Two cubic centimeters of water was added and the white crystals collected on a filter. The substance melted at 79°. The mixed melting point with the methyl sultam (VII) was 79–80°.

Summary

The sultam of 2-(N-methylamino)-*d*-camphane-10-sulfonic acid was synthesized by alkylation of the sultam of 2-amino-*d*-camphane-10-sulfonic acid which was obtained by catalytic reduction of *d*-camphor-10-sulfonanhydramide. The sultam was hydrolyzed to 2-(N-methylamino)-*d*-camphane-10-sulfonic acid. The latter was shown to be identical with the α-form obtained by catalytic reduction of 2-(N-methylimino)-*d*-camphane-10-sulfonic acid. This α-form was converted to the N-methyl sultam by acetic anhydride.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Determination of Dissolved Oxygen by Means of the Dropping Mercury Electrode, with Applications in Biology

BY HAROLD G. PETERING AND FARRINGTON DANIELS

The measurement of respiration constitutes but one of the many demands for an accurate and rapid method for the quantitative determination of oxygen. The differential pressure manometer such as the Warburg–Barcroft manometer is widely used for this purpose. The Winkler process is another standard method. The results of this investigation show that for many purposes the concentration of dissolved oxygen can be determined with great simplicity, accuracy, and rapidity by means of the dropping mercury electrode.

The method described here is a modification of the polarographic method, shown by Vitek[1] to be applicable to the determination of oxygen. The recording features of the polarograph are dispensed with and the apparatus is constructed from ordinary laboratory materials in such a way that readings of oxygen concentration in water or other conducting solvents can be made in a few seconds. It is especially useful for studying systems which involve a changing oxygen concentration.

This method may be regarded merely as an empirical analytical procedure which has been checked against chemical standards. On the other hand, theoretical interpretations of the polarographic method have been proposed.

(1) Vitek, *Chimie et Industrie*, **29**, 215 (1933); *Coll. Czech. Chem. Comm.*, **7**, 537 (1935).

Theory

The use of the dropping mercury electrode for analytical purposes depends on the fact that solutes (electrolytes or non-electrolytes) are reduced (or oxidized) when a current is passed through the solution at voltages above the decomposition potential. The current flowing de-

pends on the rate of diffusion of the material to the electrode, and this in turn depends on the concentration. The dropping mercury electrode gives reproducible results because a fresh surface of the electrode is being exposed continuously. At low voltages a small residual current flows, and then as the voltage is increased above the decomposition potential the current depends directly on the voltage in accordance with Ohm's law. As the voltage is still further increased, a point is reached after which the current is limited by the rate of diffusion of the reducible substance into the surface of the dropping mercury cathode. The current–voltage curve then becomes flat in the ideal case, and the current remains constant and independent of the voltage until the voltage becomes high enough to produce some other chemical reaction at the electrode.

The dropping mercury electrode was originally used by Kucera[2] to measure the interfacial tension of polarized mercury. Heyrovsky[3] is responsible for its extensive use in analytical chemistry. He devised the sensitive, self-recording polarograph which enables one to obtain easily and accurately the current–voltage curves of any dissolved reducible (or oxidizable) material—electrolyte or non-electrolyte. The decomposition potentials corresponding to breaks in the curve are used for qualitative analysis and the magnitude of the current at the "waves" is used for quantitative analysis.

There are several complications in the quantitative determination of a solute by this method. Other materials which have decomposition potentials in the same voltage range as oxygen will also give diffusion currents. As in any analytical procedure, these materials must be eliminated or corrected for. In this case, the readings are calibrated with a chemical method which is specific for oxygen, or these extraneous materials are determined when oxygen is removed by bubbling hydrogen through the cell. Again, these materials do no harm if only differences in oxygen concentration are being determined. When electrolytes are being determined the migration current of the ions affects the observed galvanometer readings, but this difficulty is removed by adding an excess of an indifferent electrolyte[4] such as potassium chloride (about 0.1 mole per liter). In the case of oxygen, which is a non-electrolyte, this migration current is not a factor.

Another complication is due to the (electrostatic) adsorption of reducible molecules on the surface of the mercury, which then causes a current to flow which is greater than the limiting current set by the diffusion rate. This phenomenon is evident in the maximum shown in the dotted line of Fig. 1A. These maxima are reproducible, but they may not be proportional to the reducible matter in solution, and it becomes necessary to eliminate them by the addition of small amounts of adsorbable material which are not reduced at the given potentials. Materials used to suppress these maxima are colloids such as proteins and carbohydrates, polymers, soaps, dyes, and alkaloids.[5] The adsorption of these materials tends to prevent the adsorption of the reducible material, and it does not affect the readings because they have a different decomposition potential than the material in question. It has been found in this investigation that for the analysis of oxygen there is sufficient material of this type in most biological samples and even in water which has been passed through filter paper to eliminate or greatly suppress the adsorption maximum of oxygen. The elimination of the objectionable maximum by the addition of algal cells is shown in Fig. 1B, while

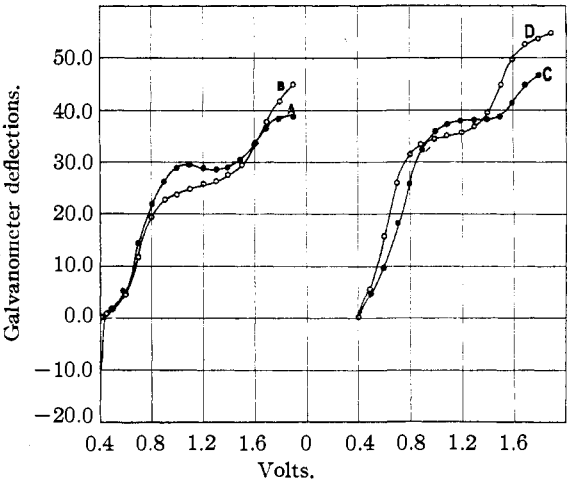

Fig. 1.

(2) Kucera, *Ann. Physik*, **11**, 529 and 698 (1905).

(3) Heyrovsky, *Phil. Mag.*, **45**, 303 (1923); *Trans. Faraday Soc.*, **19**, 692 (1923).

(4) For a discussion of the migration current, *cf.* Ilkovic, *Coll. Czech. Chem. Comm.*, **6**, 498 (1934).

(5) This subject is fully treated by Heyrovsky in "Actualités scientifiques et industrielles," No. 90, Hermann et Cie, Paris, 1934. It has been found in this work on dissolved oxygen that the alkaloids do not lead to the same diffusion current as the colloids do when the maximum is suppressed. Gelatin and ethyl cellulose are good agents for suppressing the maximum.

the dotted line A for a smaller concentration of algal cells shows that the maximum has been only partially suppressed.

As already stated, oxygen was determined with a polarograph by Vitek.[1] He showed that the absorption coefficients of oxygen in water, ethanol, and methanol, were proportional to the wave heights (*i. e.*, diffusion currents corresponding to the flat parts on the polarographic records) for oxygen in these three solvents. He gave the precision of the method as 4% and the range of dissolved gas capable of being measured as 0.04 mg. per liter up to saturation, but he did not report quantitative measurements on the absolute concentrations of oxygen. No further application of the method seems to have been published since that time.[6]

The object of the present research has been to simplify the method and to show how it can be used to advantage in a number of problems, particularly to those of biology. The simplification consists in dispensing with the recording features of the polarograph and in determining the current only at two voltages which have been determined previously by experiment.

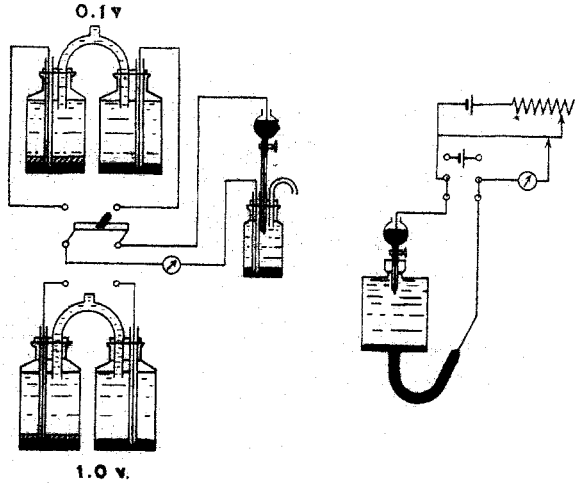

Fig. 2.

According to present practice, quantitative analysis by means of the dropping mercury electrode involves the determination of the whole current–voltage curve either by graphic or by automatic recording as in the polarograph. In some cases the difference in currents between two adjacent flat parts of the curve is taken as a measure of the concentration of the material whose decomposition potential falls in this range. In another method, parallel tangents are drawn at a definite angle above and below the region of the diffusion current, and the difference in current between the points of tangency is taken as the significant diffusion current which is proportional to the concentration.[7] The interpretation of the polarograms is fully discussed by Hohn.[8] It is much simpler, however, to determine the current at a predetermined voltage slightly less than the decomposition voltage of the electrode reactants, and again at a second voltage above the decomposition potential in the region of the diffusion current but below the decomposition voltage of a second electrode reaction. The difference between the currents flowing at these two voltages is proportional to the concentration of the substance (oxygen) taking part in the first electrode reaction. The results of this investigation show that this procedure is adequate, provided that the voltages are predetermined by experiment and the calibration curve is shown to be reproducible. The experimental apparatus then becomes extremely simple.

Apparatus

The apparatus, shown in Fig. 2B, consists merely of a stream of mercury dropping from a capillary tube into a solution in a closed bottle. The mercury stream is made the cathode and the pool of mercury the anode in a circuit which contains a storage battery, adjustable resistance and a galvanometer. The potential is varied by means of the resistance and measured with a simple potentiometer. The galvanometer need not be of the most expensive type, a sensitivity of 5×10^{-7} amp. per scale division being sufficient. A portable box type with a light and scale will do if the scale is long enough (6–10 cm.).

Since a study of the whole current–voltage curve showed that only two voltages are necessary for the analysis, it is evident that even the potentiometer may be dispensed with and two large capacity standard cells substituted for it as shown in Fig. 2A. The standard cells are made from two eight-ounce square bottles taped together, closed with rubber stoppers, and connected with a salt bridge. Connections are made with the mercury or amalgams, at the bottom of the bottles, by means of platinum wire fused to copper wire and sealed into glass tubes fitting into the stoppers. In the 1.0-v. Weston cell one bottle contains 100 g. of 13% cadmium–mercury amalgam. (The cadmium dissolves more quickly if its surface is in-

(6) After this manuscript was completed it was learned that Baumberger and O. H. Müller reported an application of the polarographic method to respiration measurements at the meeting of the Western Society of Naturalists in 1935.

(7) Borcherdt, Adkins and Meloche, THIS JOURNAL, **59**, 2171 (1937).

(8) Hohn, "Chemische Analysen mit dem Polarographen," Verlag von Julius Springer, Berlin, 1937.

creased by cautiously pouring the molten metal into water.) The other bottle contains about the same amount of pure mercury covered with a paste of mercurous sulfate in 2 molar cadmium sulfate. Both bottles are then filled to the top of the bridge with approximately 2 molar cadmium sulfate and the opening at the top of the bridge is closed. Such a cell maintains a constant potential of about 1.04 v. over long periods of time and is unaffected by ordinary currents during continued usage.

The 0.1-v. cell is a cadmium–lead cell[9] in a similar double-bottle. One electrode contains 10% lead in 90% mercury over which stands a solution of about 1 molar cadmium iodide saturated with lead iodide. The other electrode contains 11% lead, 9% cadmium and 80% mercury in a solution of about 1 molar cadmium iodide.

As shown in Fig. 2A a double pole-double throw switch is used for throwing in first the 1.0-v. cell and then the 0.1-v. cell. The difference in galvanometer readings with the two cells gives the desired measurement which is proportional to the concentration of dissolved oxygen.

The dropping mercury cathode must be adjusted carefully to give maximum galvanometer deflections with minimum oscillation and without clogging of the capillary tube. A separatory funnel is connected with a capillary tube from a broken thermometer through a minimum length of pure gum rubber tubing or rubber which has been boiled with sodium hydroxide to remove sulfur. The rubber tube is wired on and reinforced if necessary with a wrapping of tape. A 50-cm. head of mercury is satisfactory and the tip is so made that the mercury drops off at the rate of about one drop every one or one-half seconds.

Several different types of cells were used in this work. The cells are completely filled with the liquid, leaving no gas space. In one type (Fig. 2A) the solution overflows as the mercury flows in. In another type (Fig. 2B) the mercury pool at the bottom is kept at a constant level, by allowing the mercury to overflow. In still another type the anode was a calomel electrode connected through a bridge of potassium chloride (1.0 *N*) solution. In the photochemical work the sides of the cell were made of polished glass and care was taken to place the mercury cathode in the center of the cell.

Calibration

In using the dropping mercury electrode it is necessary to calibrate the galvanometer readings directly against the concentration of the substance being analyzed under the same conditions, and in the simple method proposed here it is necessary first to determine empirically at what voltage the galvanometer readings should be taken in order to give a straight line when the concentration is plotted against deflections. Using the same tip and the same apparatus, then the concentration of an unknown solution is obtained readily by interpolation on this straight line or by simply multiplying the galvanometer readings by a constant, *i. e.*, by the slope of the calibration line.

In Fig. 3 are shown current–voltage curves at 18° for various concentrations of oxygen in a solution containing some suspended algae. The full curve is shown at a concentration of 3.00×10^{-4} mole per liter while for the other concentrations the range only from 0.7 to 1.0 v. is shown. When the currents at 0.7, 0.8, 0.9, 1.0 v. are plotted against the concentration of oxygen, only the points at 1.0 v., marked with double circles, give a straight line, as shown in A of Fig. 4. It is clear from an inspection of Fig. 3 that if a straight line is produced at 1.0 v., a straight line cannot be produced at the other voltages.

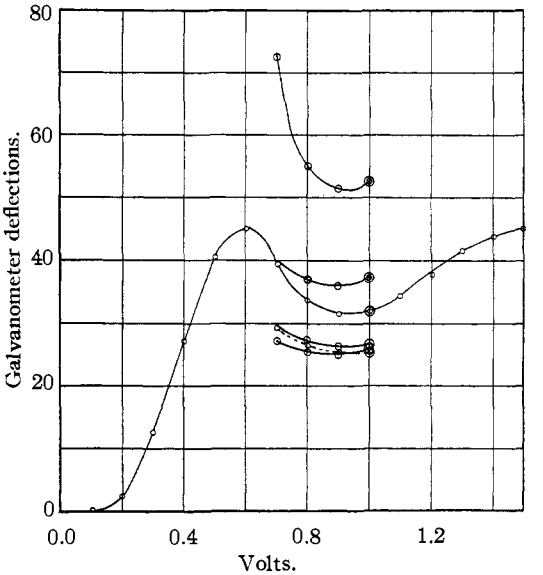

Fig. 3.

In these calibrations the oxygen concentration was determined by titration with sodium thiosulfate using the standard Winkler method[10] with manganous sulfate and potassium iodide. The oxygen concentration was controlled by equilibrating the nutrient solution in which the chlorella were suspended (or just the salt solution, for example 0.1 *N* potassium chloride) with air at the specified temperature, and then passing through this solution oxygen, or nitrogen, or gases containing some oxygen. The closed vessel, containing the solution with dissolved oxygen, was fitted with a siphon, and the samples were withdrawn quickly for chemical analysis and for analysis with the dropping mercury electrode under conditions giving a minimum of change in the oxygen concentration.

Four different calibration curves (A, B, C, and D) obtained by the first method are shown in Fig. 4. These curves illustrate the necessity for separate calibrations for each tip and for each temperature as well as for each galvanometer and electrical circuit. Calibrations A, B, and C were all made with the same dropping mercury cathode tip, but at different temperatures—namely, 18, 22, and 28°, respectively. Calibration D was made with a different tip and at 25°.

An examination of these calibration curves shows the high sensitivity of this method of analysis. Assuming that the galvanometer can be read to at least one-half of a scale division (0.5 mm.), the corresponding limit of error in the oxygen concentration is of the order of 5 ×

(9) Vosburgh, THIS JOURNAL, **49**, 2223 (1927).

(10) Treadwell and Hall, "Analytical Chemistry," Vol. II, 7th edition, John Wiley and Sons, Inc., New York, 1928, p. 650.

10^{-7} mole per liter. The experimental points fall on the straight line within these limits. It is quite convenient to use the dropping mercury electrode with samples of about 10 cc.; and for a sample of this size the actual change which can be measured is of the order of 5×10^{-9} mole total change in oxygen concentration. At room

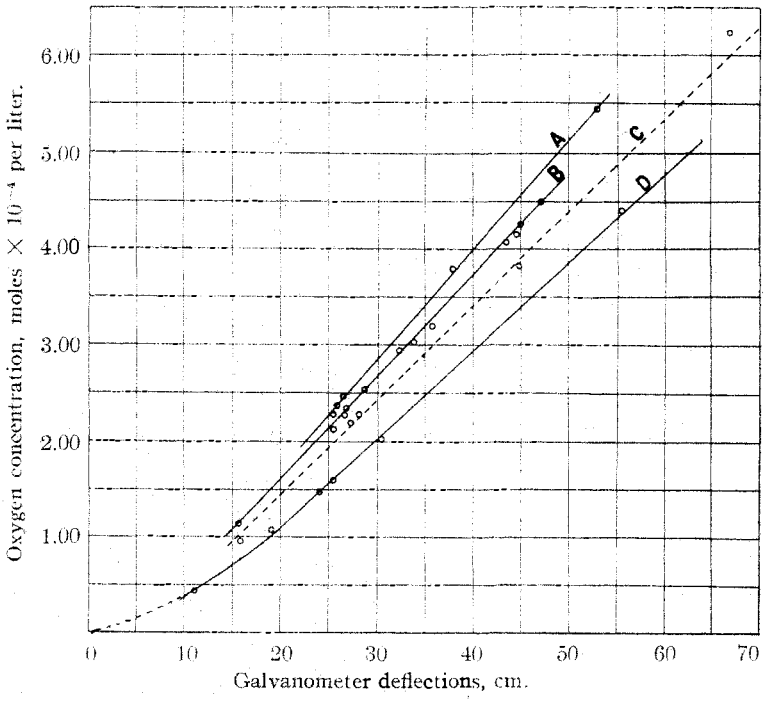

Fig. 4.

temperatures this corresponds to 1.2×10^{-4} cc. or to 0.112 cu. mm., or 1.6×10^{-7} g. Expressed in another way the dissolved oxygen can be determined to 0.016 part per million by weight. These estimates of the sensitivity of the method are substantiated by the curves and particularly the data of Table I.

A simple calculation shows that the passage of current during the reading of the galvanometer deflections is far too small to cause detectable change in concentration by electrolysis.

The straight line relation between concentration and current apparently fails to hold below a concentration of about 5×10^{-5} mole per liter. The straight lines do not extrapolate to zero current at zero concentration, an observation which has been discussed by several investigators. In curve D of Fig. 4 special effort was made to determine the relation between concentration and current in this region. The Winkler method loses its sensitivity at these low concentrations and hence the true situation is difficult to determine. The curve bends toward the axis so that it extrapolates to zero, and this fact has been checked by other determinations. Despite this complexity, it is possible to use the method outlined here for low concentrations of oxygen by interpolating on the curve even where the straight line relationship does not hold.

The limitations of this simplified method should be summarized. The substitution of two empirically determined voltages for the complete polarogram is possible only after a complete voltage–current curve has been plotted, and the proper voltages checked by independent chemical or physical determinations of the concentration of dissolved oxygen. However, after these voltages have been established it is no longer necessary to determine these complete curves. The preliminary curves can be prepared, manually, with simple apparatus. Calibration must be carried out for each cell and tip, and for each new type of solution unless it can be shown that no interfering substances are present. However, such calibrations are necessary, also, in the complete polarographic method. Calibration can be effected by direct chemical analysis as with the Winkler method, or by removal of oxygen by the bubbling of hydrogen and the addition of measured quantities of oxygen. It might be argued that, with this calibration method, a single voltage rather than two voltages would be sufficient, but the zero point of the galvanometer fluctuates with temperature and accidental variations in the amount of iron and other easily reducible substances give large fluctuations in the galvanometer deflections even at 0.1 volt. The fluctuations are cancelled out by taking the difference between two voltages, 1.0 and 0.1 v., for example, when analyzing for oxygen. The maximum deflections of the galvanometer are taken at a given voltage and these maxima are read easily when the mercury is dropping at the rate of one drop every second or second and a half. Although this rate may be considered somewhat rapid for producing a "theoretical" curve, it has been shown to be entirely satisfactory for the method described here.

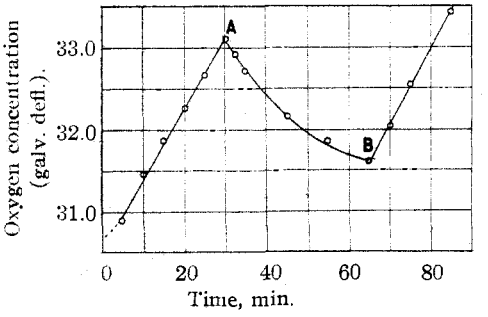

Fig. 5.

Applications

The accuracy and the quick response of this method is clearly shown in Fig. 5 where the photosynthesis of algae is protrayed. In this experiment light from a 500-watt projection lamp was filtered through 9 cm. of 0.025 *N* cupric sulfate and a Corning No. 243 filter. This red light at

about 3500 ergs per second per sq. cm. was passed into a vessel containing *chlorella pyrenoidosa* suspended in Warburg nutrient solution, 16,000,000 cells per cc. The steady evolution of oxygen is indicated by the straight line with a positive slope. At point A, the light was turned off and photosynthesis stopped, but the respiration of the algae continued. The negative slope shows that oxygen is being consumed by the respiration. It is of interest to note that the rate of respiration, *i. e.*, the slope of the line, is greatest just after removal of the light. It was, in fact, to study this rate of respiration just after the exposure to light that this method was developed. Chemical analysis and differential manometric methods have failed to give information of any degree of definiteness or consistency about this rate of respiration immediately after exposure to light. When the light is turned on again at B, the photosynthesis immediately releases oxygen and the net effect of respiration and photosynthesis is a uniform increase in concentration of oxygen as before. The slopes of the oxygen concentration–time curves during exposure to light are identical within experimental error.

Before using this method it was necessary to prove that mercury is not toxic to chlorella—at least not in the concentration to which this method subjects the algae. Algae were cultured in the presence of 1 cc. of purified mercury of the grade used in the dropping mercury electrode. The algae grew normally along with many controls which were a part of the regular culture stock maintained for experimental purposes. No differences were noticed in the appearance of the algae grown in the presence of and in the absence of mercury, nor was there any noticeable difference in the total growth at the end of seventeen days. The algae which were grown in the presence of mercury respired and photosynthesized in the same way as those which were grown in the absence of mercury. Likewise, Mr. Albert E. Dimond of the Botany Department, who furnished the yeast used in some of these experiments, found that yeast is not affected by the presence of the mercury in these experiments.

Other applications of the analysis of oxygen by the dropping mercury electrode method are given in Fig. 6. Curves A, B, C, and D are for the respiration of yeast. They show how the respiration rate increases with the concentration of cells and with the addition of glucose. Curve E is for

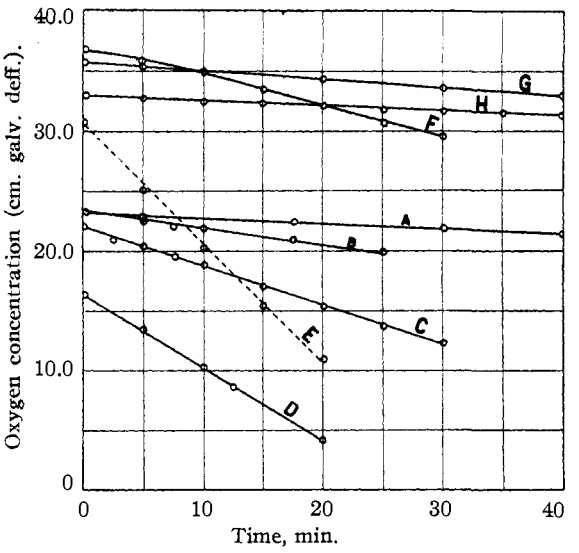

Fig. 6.—A. Yeast in physiological salt solution—3 × 10^6 cells/cc. B. Yeast in physiological salt solution + 1% glucose—3 × 10^6 cells/cc. C. Yeast in physiological salt solution + 6% glucose—3.3 × 10^6 cells/cc. D. Yeast in physiological salt solution + 6% glucose—6.6 × 10^6 cells/cc. E. Homogenized liver tissue in Krebs phosphate buffer + sodium succinate. (7.13 g. rat liver + 400 ml. buffer solution + 0.5 g. Na succinate.) F. Chicken's red blood cells—Ringers bicarbonate buffer—9 × 10^7 cells/cc. G. Chicken's red blood cells—Ringers bicarbonate buffer—3 × 10^7 cells/cc. H. Dog's red blood cells—Ringers bicarbonate buffer—60 × 10^7 cells/cc.

the oxygen uptake of homogenized rat liver tissue in the presence of sodium succinate four hours after the rat had been killed. The volt–current curve for this material is shown in Fig. 1, D. Curves F and G give the respiration rates of red blood cells from chickens. F and G are for the same cells, but G was obtained with a suspension in which the concentration of cells is about one-third that of F. Curve H shows the respiration of the red cells of dog's blood in vitro and it is particularly interesting because the respiration is so slight that it can be followed by other methods only with great difficulty. Accordingly, the de-

TABLE I

RESPIRATION OF DOG'S BLOOD

60 × 10^7 cells per cc. Temperature, 24.5°. According to the calibration of the electrode 1-cm. deflection = 3.88 cu. mm. of O_2. Over-all uptake of O_2, 7.56 cu. mm. Average uptake of O_2, 0.84 cu. mm. in five minutes.

Time, min.	Galvanometer deflection, cm.	Time, min.	Galvanometer deflection, cm.
0	33.10	25	31.95
5	32.90	30	31.75
10	32.60	35	31.70
15	32.40	40	31.45
20	32.20	45	31.15

tails of the experimental measurements are given in Table I and the volt–current curve for the dog's blood is shown in Fig. 1 at curve C.

When the chicken and dog blood are put on the same basis of concentration of cells, it is seen that chicken blood takes up in thirty minutes twenty times as much oxygen as the dog blood. The cells of the chicken bloods are nucleated while those of the dog blood are not.

Measurements were made on the dog blood cells at the same time using a Barcroft–Warburg manometer. However, the oxygen uptake was too small to be measured with any accuracy by the manometric method.

Another application of the method described here lies in the determination of the oxygen content of soils. This analysis has been beset with many difficulties and the values which have been obtained have little significance according to many workers in soil culture. The methods described here may be used, by determining first the oxygen content of a given volume of 0.1 *N* potassium chloride solution, and then determining the oxygen content after adding a sample of soil and allowing equilibrium to be established. In one sample of loam, 33 cu. mm. of oxygen was found to be associated with 1 g. of soil with a moisture content estimated as 30%.

The apparatus is portable and it can be made adaptable to field use such as determining the oxygen content of lakes at different depths.

There are other fields of study in biology which might be attacked with this precise method of oxygen analysis such for example as the study of the border line between aerobic and anaerobic bacterial action, the respiration of small amounts of tissue and nerve material, the accurate measurement of the respiration of cancer cells and the study of the influence of drugs and various diseases on the respiration of blood.

Although this investigation has been limited to the determination of dissolved oxygen, it is likely that other substances can be determined in the same simple way. Calibration curves with galvanometer deflections plotted against concentration are plotted at several different voltages and that voltage (or rather the difference in current between two voltages) is selected which gives a straight line for a calibration curve. Aside from giving the confidence which results from a straight line relationship over a wide range of concentration, this method renders unnecessary any expensive recording apparatus and enables one to make an analysis on systems undergoing rapid chemical or biological change.

The authors are glad to acknowledge the support given this investigation by the Research Committee of the Graduate School and the Wisconsin Alumni Research Foundation. They are indebted to Professor B. M. Duggar of the Botany Department in whose laboratory much of the work was done. They wish to thank Professor V. W. Meloche for his helpful interest and for the use of a polarograph in a preliminary test. They appreciate the help given by Mr. A. Axelrod in the measurements with the Barcroft manometer and by Mrs. N. Dimond in preparing the algal cultures.

Summary

1. A method using simple apparatus is described for measuring the concentration of dissolved oxygen. A dropping mercury electrode is used and galvanometer deflections are measured at two predetermined voltages. The deflections are plotted against solutions of known concentrations giving a calibration curve which is a straight line.

2. The limitations of the method are pointed out and the method is illustrated with the photosynthesis and respiration of algae and with the respiration of yeast and blood cells and with animal tissue.

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