OXYGEN TENSION MEASUREMENT BY A METHOD OF TIME SELECTION USING THE STATIC PLATINUM ELECTRODE WITH ALTERNATING POTENTIAL

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There has been a well recognized need for an adequate method of measuring oxygen tension in small volumes during short time intervals without disturbing movement and without toxicity to living cells. Conventional polarographic technique provides the kind of data desired, but its dropping mercury electrode obviously fails to meet the requirements.

The static platinum electrode has been used in polarographic analysis (Laitenen and Kolthoff, 1942) but a number of difficulties are encountered: (1) the long time required for observation, (2) instability, and (3) lack of reproducibility. In biological application of this electrode, Davies and Brink (1942) reported a day-to-day variability of 30 per cent and Roseman, Goodwin, and McCulloch (1946) interpret only relative values for oxygen. The recessed electrode used by Davies and Brink improves the stability and reproducibility, but imposes a long time for restoration between successive observations.

The rotating platinum electrode employed by Laitenen and Kolthoff (1942), though improving stability, not only introduces disturbing movement but lacks reproducibility. In like manner the flow system of Giguère and Lauzier (1945) imposes similar restrictions.

In this investigation the possibility has been explored of overcoming the difficulties attendant on the use of the open, static platinum electrode through the employment of a suitable pattern of alternating potential with techniques arising from a special consideration of the time aspects involved. Because a good many other investigators have considered the possibility of an alternating potential, our observations over a wide range of frequency and pattern will be reported as well as the choice which has yielded the most satisfactory results.

Since the shortest possible time between successive measurements was desirable our observations began at 30 C.P.S.[1] and were extended ultimately to quite low frequency, 6 to 12 C.P.M.[2] Since these observations developed

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[1] Cycles per second.

[2] Cycles per minute.

information essential to an understanding of the phenomena and the inherent limitations encountered and although they are preliminary in character they will be reported in the order of development. Initially a square wave potential pattern was selected for study, largely because of greater ease of interpretation. Subsequent findings support this as a desirable choice.

Oscilloscope Observations

Potential wave patterns were first produced by a photoelectric type wave generator in which two sector discs cut to produce the desired wave form were rotated between the light source and each of two photo tubes operating through a single amplifier. The pattern of potential thus produced was imposed on an electrolytic cell composed of a platinum electrode of 20 gauge wire about 3 mm. long, *versus* a M/10 calomel half cell both immersed in a solution of M/10 KCl. An external resistance was introduced in series with the cell and the current pattern observed by following the potential difference across this resistance by means of a Dumont type 208B oscilloscope. A dual electrolytic cell was used as a means of comparing electrode performance in oxygen- and nitrogen-saturated KCl solutions. The square wave pattern chosen (Fig. 1) included a shorting period of no applied potential between each pulse, providing a "rest" interval during which the charge accumulated during the previous pulse is largely dissipated. Typical resulting current patterns for a frequency range of 5 to 30 C.P.S. at diffusion limiting potentials are shown in Fig. 2. These current patterns are repeated from cycle to cycle and appear stable on the oscilloscope screen.

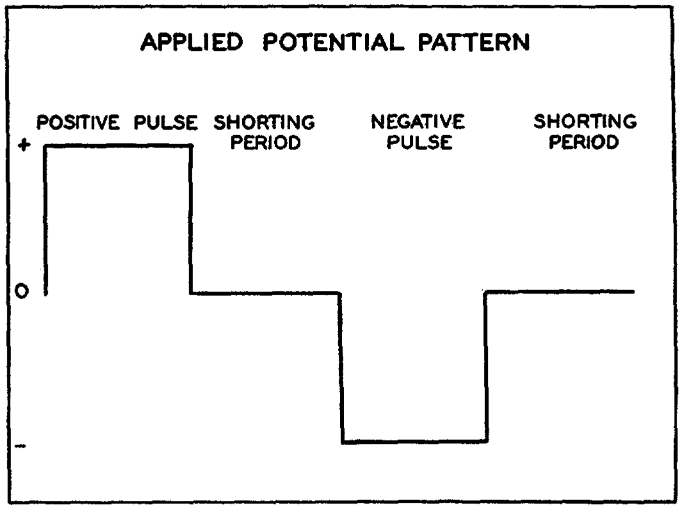

FIG. 1. See text.

It will be noted that greater influence of oxygen occurs at the end of each pulse and the beginning of each shorting period than elsewhere in the cycle, and that of the negative pulse slightly exceeds that of the positive. It can be seen from Fig. 3 that this influence of oxygen on the negative pulse terminal current increases with diminishing frequency in the range of 14 to 2.5 c.p.s. (pulse duration of 0.0178 to 0.1 second). This is not the case for the positive pulse terminal current as seen in Fig. 4. Fig. 3 shows that even with a pulse duration of 0.1 second there still exists a considerable (10 per cent) deflection for tank nitrogen which cannot be attributed to its O_2 content of about 1½ per cent. In view of the trend, both considerations suggest continuing to still lower frequencies. This required a change in technique since the lower limit of flat frequency response of the oscilloscope used was about 2.5 c.p.s.

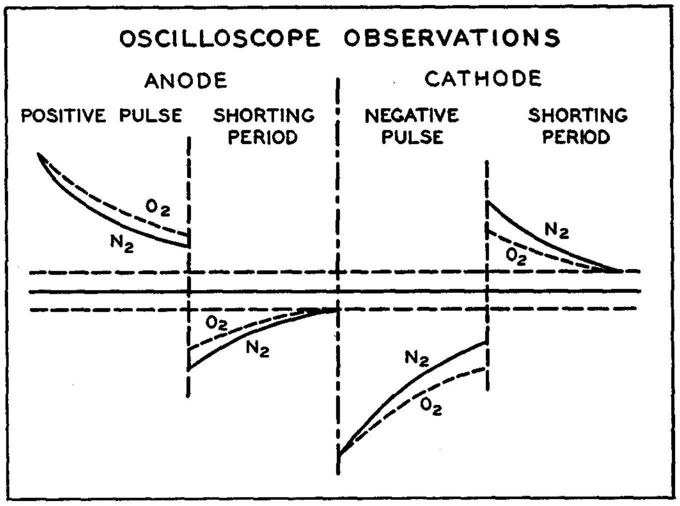

FIG. 2. Typical current patterns for oxygen- and nitrogen-saturated solutions when the potential pattern of Fig. 1 is applied in the frequency range of 5 to 30 c.p.s.

Oscillograph Galvanometer Observations

Subsequent observations at lower frequencies were made with Westinghouse "ultrasensitive" galvanometers adapted for continuous photographic recording. They provide a flat frequency response from 1500 c.p.s. to D.C. and a sensitivity of 100 μa/inch when used with an optical lever of 15 inches. These are oil-damped galvanometers of the moving permanent magnet type with two field coils. In order to secure adequate deflection a single stage push-pull D.C. amplifier with an input resistance of about 25,000 ohms was introduced.

Since a more stable zero and more accurate potential pattern than those obtained with the photoelectric type wave generator were desirable a cam-driven microswitch arrangement was substituted. This produced the same square wave potential pattern described previously.

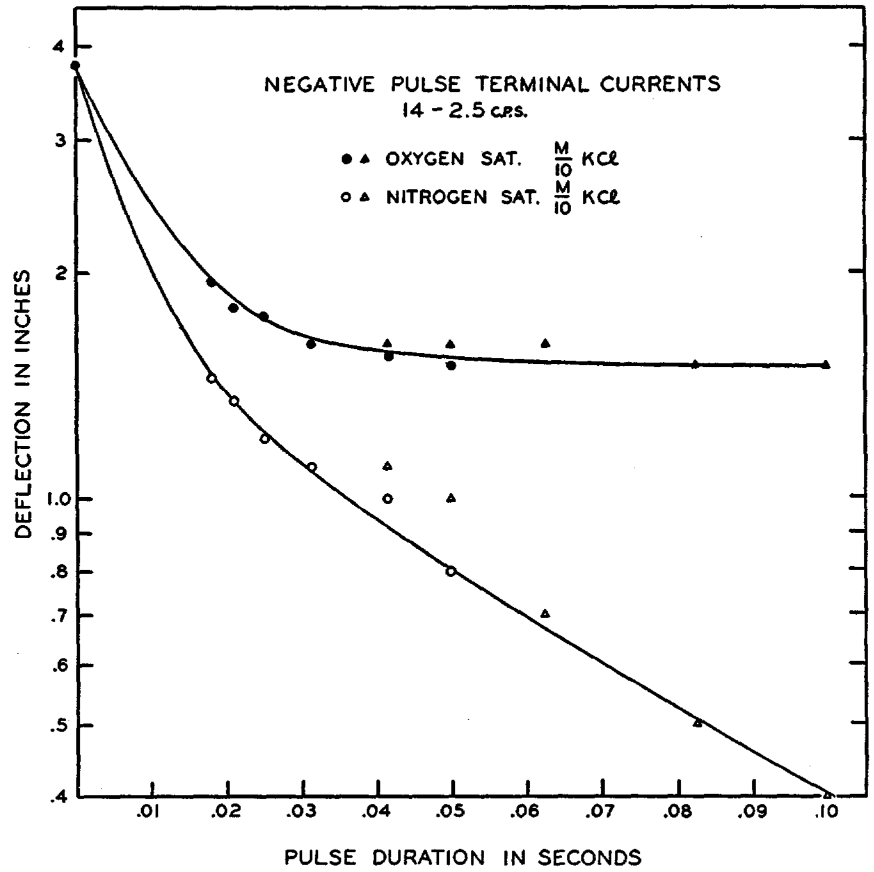

Fig. 3. Effect of pulse duration on negative pulse terminal currents due to high oxygen and low oxygen for frequencies in the range of 14 to 2.5 c.p.s. Current expressed in deflection recorded from oscilloscope screen.

Significance of Current-Time Patterns.—In Fig. 5 the current-time curve during a negative pulse of 0.7 second duration (about $\frac{1}{3}$ c.p.s.) is plotted for both oxygen- and nitrogen-saturated solutions. The long linear portion shown on this semilog plot when oxygen is very low, suggests the behavior of a condenser with a time constant of about 0.4 second. Thus a simple[3] interpretation of our observations is that in the absence of oxygen we have

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[3] A more complete and rigorous consideration of the time course of current will be presented in a later article by one of us.

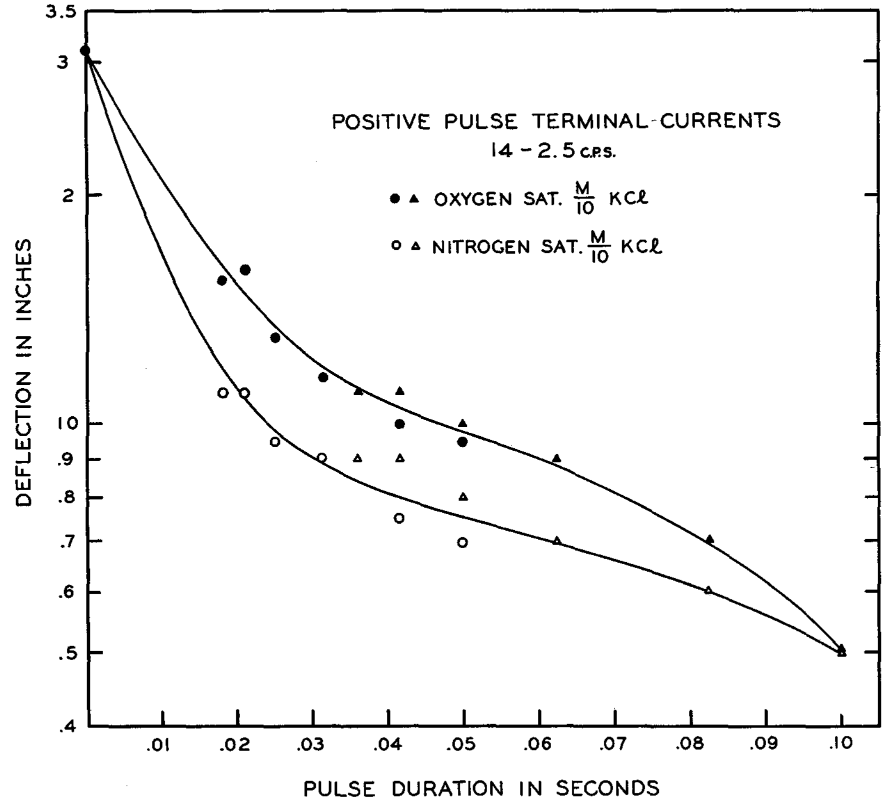

FIG. 4. Effects of pulse duration on positive pulse terminal currents for high and low oxygen.

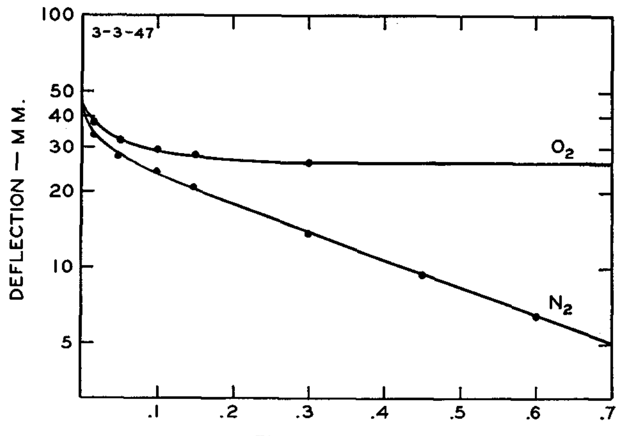

FIG. 5. Current-time curves during a negative pulse of 0.7 second duration (about ⅓ C.P.S.) in oxygen-saturated and nitrogen-saturated M/10 KCl solutions. Current expressed in millimeters of deflection of oscillograph galvanometer.

during each positive and negative pulse the simple charging of a capacitative surface, followed during the shorting phase by the discharge of this surface. The presence of oxygen permits a conduction through the surface resulting in a sustained current during each pulse and a corresponding reduction in the discharge current during the subsequent shorting phase.

After a longer pulse duration the oxygen influence on the positive phase disappears, whereas it is enhanced in the negative phase. To avoid the background current in the absence of oxygen, if correctly interpreted as a capacitative charging current, three alternatives appear promising. (1) To reduce the time constant by reduction in external resistance; (2) to proceed to longer time intervals; (3) to cancel out the alternating component due to charge and discharge of the surface and measure only the rectified current component remaining chiefly from the negative pulse.

The last alternative was tested readily and resulted in observations which were satisfactory in magnitude of oxygen dependence and without background contribution in the absence of oxygen. The relation of response to oxygen concentration, however, proved to be non-linear of a character suggesting adsorption dependence; *i.e.*, very sensitive to low concentration but approaching saturation in the vicinity of 20 per cent.

A similar non-linear dependence was found for the oxygen response during the time range where there remained an appreciable background of capacitance charging current (Fig. 6).

Accordingly both remaining possibilities—reducing time constant and also lengthening the duration were explored. If the duration was made too great the instability common to the older static electrode techniques was encountered. However, reducing the time constant by cutting the external resistance below 2,000 ohms yielded satisfactory linearity of oxygen dependence for the terminal current of the cathode pulse. Typical current-time patterns recorded under these conditions are shown in Fig. 7.

Our interpretation is that with a short time constant, the capacitative current decays to a negligible value in time to observe the diffusion limited current before the diffusion wave extends far enough from the electrode to be subject to thermal convective interference and mechanical disturbances.

The initial oxygen influence is attributed to the discharge of an adsorbed layer of oxygen. Later the diffusion limitation asserts itself thus resulting in linear dependence. The discharge of the adsorbed oxygen appears to closely parallel the capacitative charging period.

This view was supported by the finding that during bubbling the deflection increased but became non-linear. Here we assume the diffusion limiting zone was swept away leaving again the adsorbed layer as the controlling factor (Fig. 8).

A family of current voltage curves for different durations are shown in

Fig. 9 with reduced external resistance. Here it will be seen that the typical diffusion limited plateau is developed for longer pulse duration and is absent for shorter durations. Thus the linearity of oxygen dependence parallels the development of the plateau. Refer to A, Fig. 7, and lower curve, Fig. 8. These findings form the basis for the final instrument to be described.

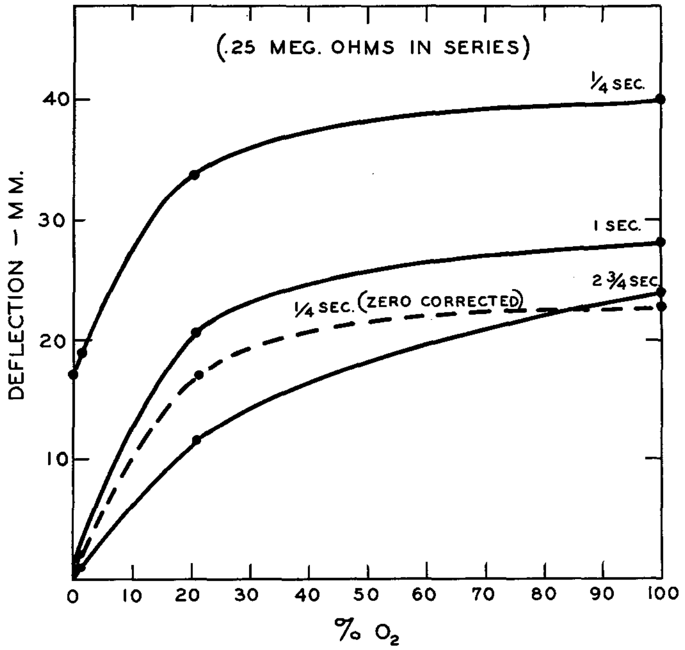

FIG. 6. Family of curves showing non-linear oxygen dependence of negative pulse terminal currents in the pulse duration range of appreciable background capacitative charging current. A resistance of 0.25 megohm was introduced into the external circuit during these observations.

Description of Present Apparatus

In the application of the electrode two types of recording are required (1) recording at increasing voltages in order to obtain current-voltage curves analogous to the typical polarogram for the identification of ions discharged, and (2) continuous recording at an appropriate voltage for diffusion limited discharge of the ion whose concentration is to be followed. Application of the preceding analysis of current-time curves requires that the current in the above cases be recorded at the end of each successive negative pulse. This is accomplished in our technique by a cam-microswitch arrangement which, in addition, produces the potential wave form. A stepping relay

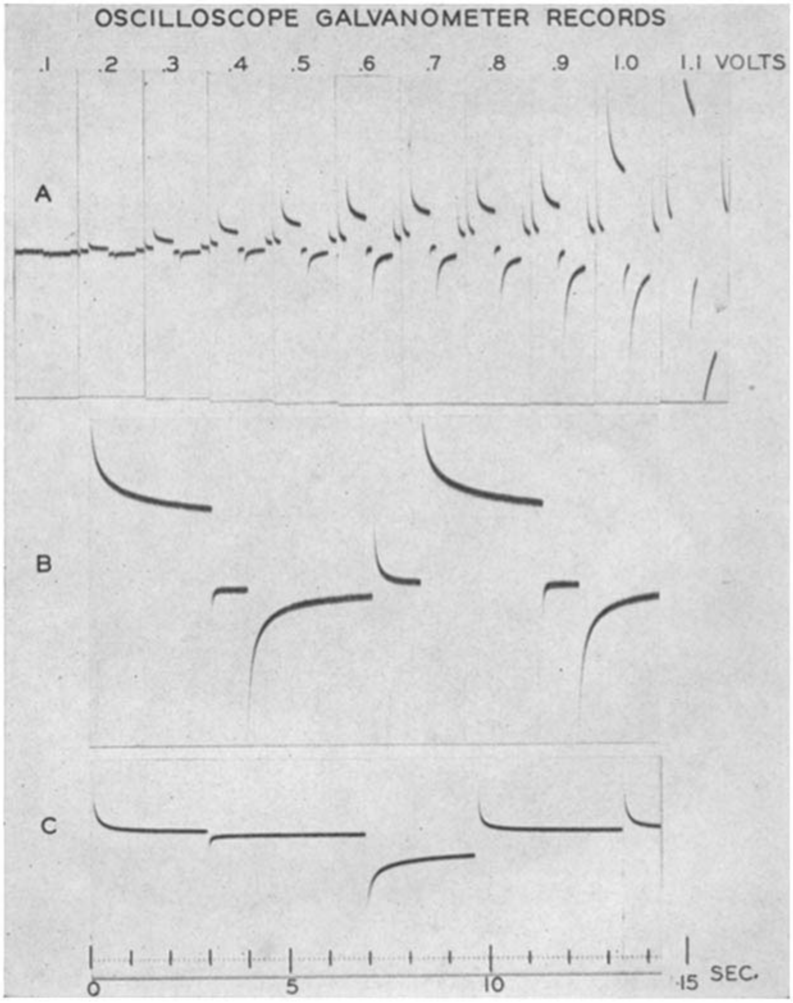

FIG. 7. Typical current-time records. *A*, composite of representative cycles for each 0.1 volt applied potential showing development of plateau. Upper pulse is negative. *B*, current-time curve in O_2-saturated solution for potential pattern with diminished shorting period (10 sec./cycle). *C*, current-time curve in air-saturated solution (lower pulse is negative).

provides a means of automatically increasing amplitude in steps for the optional recording of current-voltage curves. The circuit diagram of this instrument is shown in Fig. 10. Microswitches (single pole double throw) MS_1 and MS_2 each connected to potential sources of opposing polarity are

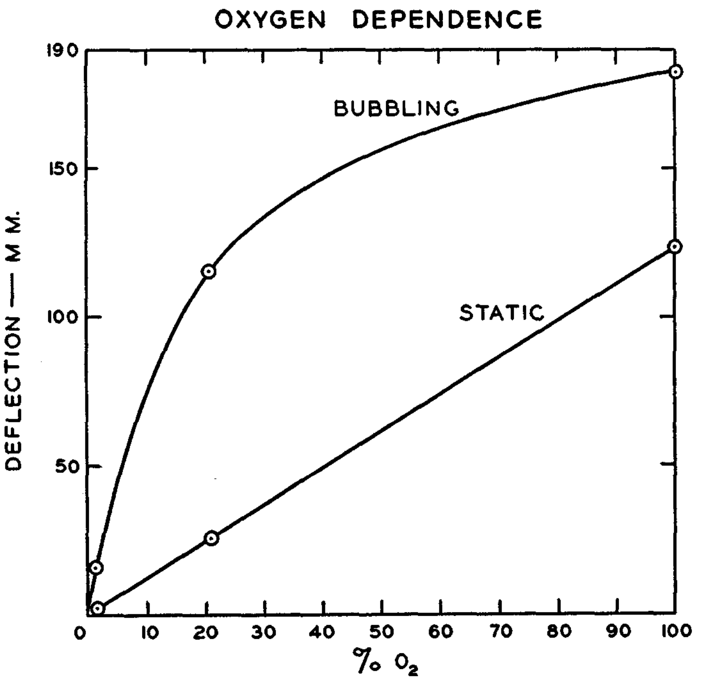

FIG. 8. Influence of stirring due to bubbling solution with gas mixtures.

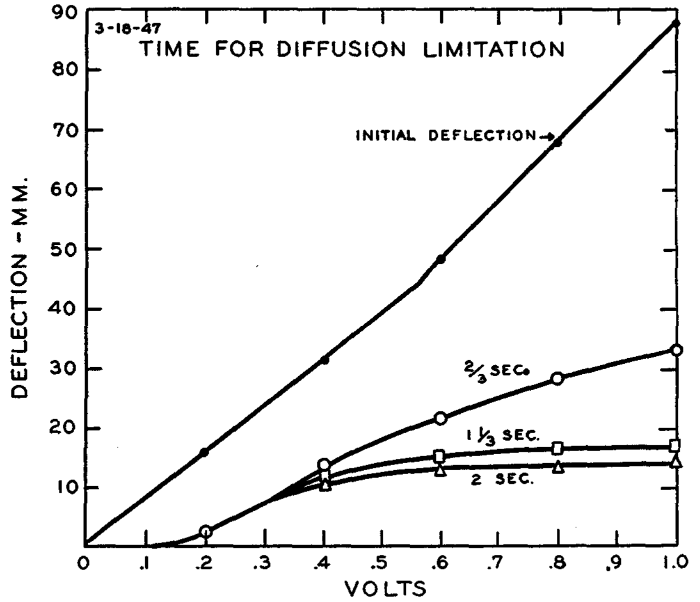

FIG. 9. Family of current-voltage curves for different durations during a negative pulse of 2 seconds in air-saturated solution showing development of the oxygen plateau with time.

placed at diametrically opposite switching positions with respect to the axis of a cam. The cam is of such a shape that each microswitch is closed during approximately a quarter of a revolution. As the cam rotates and closes MS_1 a negative pulse is imposed on the platinum electrode across the electrolytic cell. When the cam continues to revolve through the next quarter revolution and allows MS_1 to open, the cell is shorted through R_{15}, R_{13}, and R_2 to R_{12} depending on the position of the stepping switch. At the beginning of the third quarter revolution MS_2 is closed and a positive pulse is imposed on the cell. The final quarter revolution provides another shorting period and the whole sequence produces the wave form used in the preceding experiments.

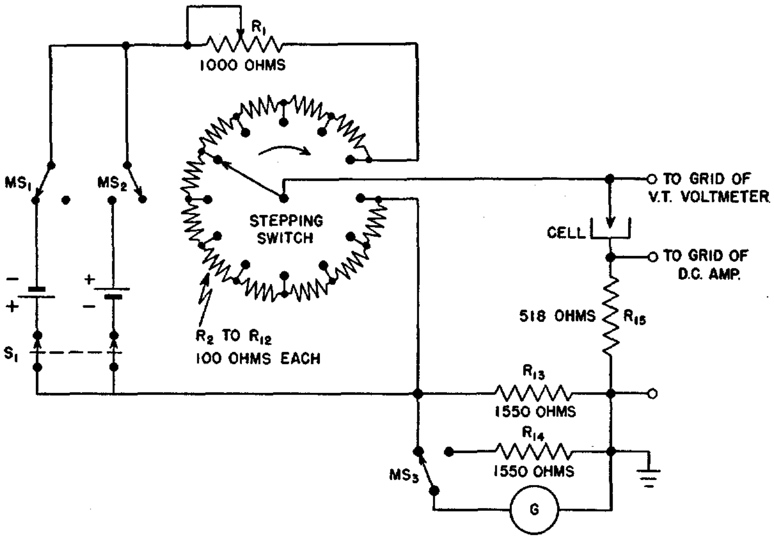

FIG. 10. Circuit diagram (see text).

In order to record only the negative pulse terminal current an additional cam is provided on the same shaft, which operates microswitch MS_3 (single pole double throw) just before MS_1 is opened. This introduces the current at the end of each negative pulse to galvanometer *G*, a Cambridge galvanometer of 0.83 second period, sensitivity 140 mm./μa (112 mm./μa with shunt) and critical damping resistance 1890 ohms, adapted for photographic recording.

The resistances R_{13} and R_{14} provide sufficient damping to allow for good registration of maximum deflection and zero values respectively. The record appears as a series of points each representing the maximum deflection or current flowing at the end of each successive negative pulse. R_{15} is provided as a load resistance for the D.C. amplifier associated with a Westinghouse oscillograph galvanometer in order that current-time patterns may be studied under various conditions of electrode, supporting solution, etc. This, however, is used in connection with further development of techniques and is not essential for routine measurements.

In order to record current-voltage curves or polarograms during the course of experiment, an automatic stepping switch is combined with precision resistance R_2 to R_{12} as a voltage divider to increase stepwise the amplitude of the applied potential wave. This stepping switch is actuated by an additional cam and microswitch combination geared to the mechanism described above.

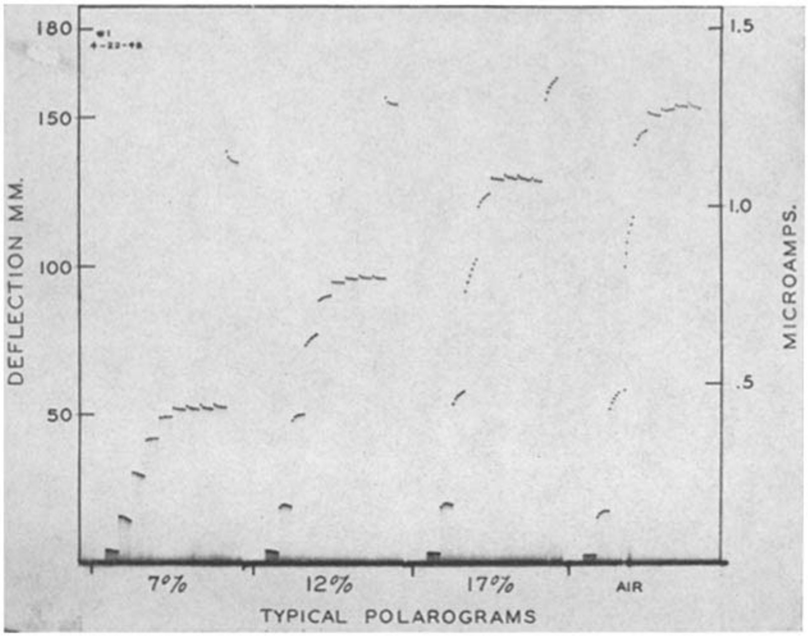

FIG. 11. Stepwise current-voltage records used in calibration. (Record for $1\frac{1}{2}$ per cent O_2 not shown.)

Since increasing the potential stepwise from zero requires several cycles (see Figs. 11 and 17) at each step before equilibrium is established the stepping cam is geared to the commutating cam in a ratio of the number of swings per step desired. At present six per step are being used and polarograms are plotted from the final swing of each step. This system avoids the manual counting of cycles and provides accurate timing of voltage increase with respect to the phase of applied potential wave. R_1 is provided in the circuit to allow for the total potential range and the degree of change per voltage step.

Electrodes.—Electrodes used at present are of 25 μ diameter platinum wire embedded in soft glass capillary drawn out to about 3 mm. This small diameter affords a better metal-to-glass bond and provides better stability than the larger type. The length of exposed platinum wire is about 4 mm.

These electrodes are incorporated with a calomel half cell in an assembly designed for concentric entry into a conventional single opening absorption cuvette of 1 cm. path as shown in Fig. 12. The salt bridge is flushed by insertion of a hypodermic needle through the hollow rubber stopper. The assembly is clamped in a metal holder arranged so that the cell may be opened and closed with proper alignment of electrode and cuvette to avoid bending or breakage of the platinum when changing solutions or suspensions. The assembly is immersed in a constant temperature bath to the level indicated in the diagram.

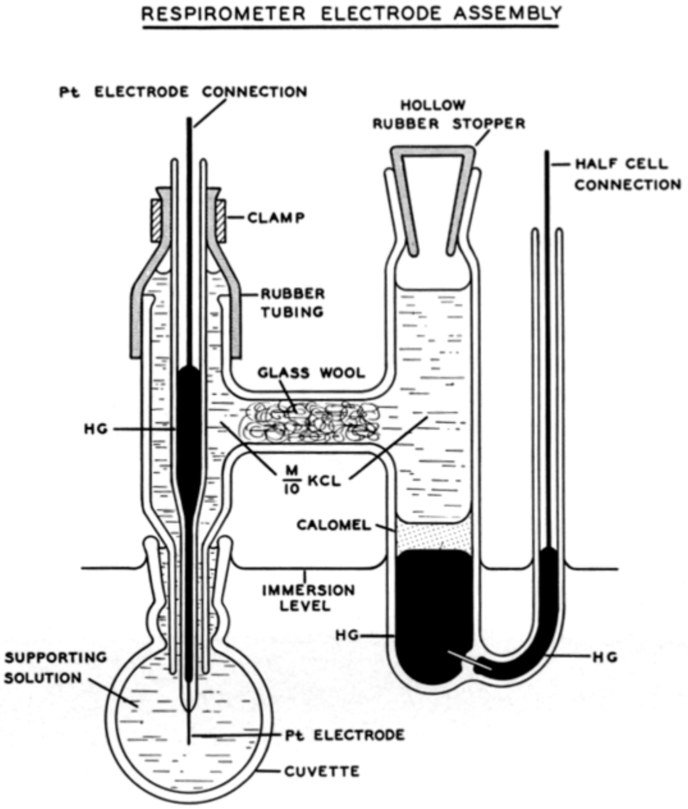

FIG. 12. Diagram of electrode assembly. Cuvette is about 15 mm. in diameter.

Calibration.—Calibration of each electrode is accomplished by measuring deflection in solutions saturated at atmospheric pressure with known oxygen-nitrogen mixtures in the range of oxygen percentage between air and pure nitrogen.[4] These solutions may be saturated simultaneously and then introduced interchangeably into the cuvette by means of a hypodermic syringe taking care to avoid contamination by room air. The needle is introduced into the bottom of the cuvette and the solution slowly expelled until it slowly displaces the air and overflows by a volume several times that of the cuvette. Nitrogen-saturated solutions showed no measurable contamination by air when carefully manipulated in this manner.

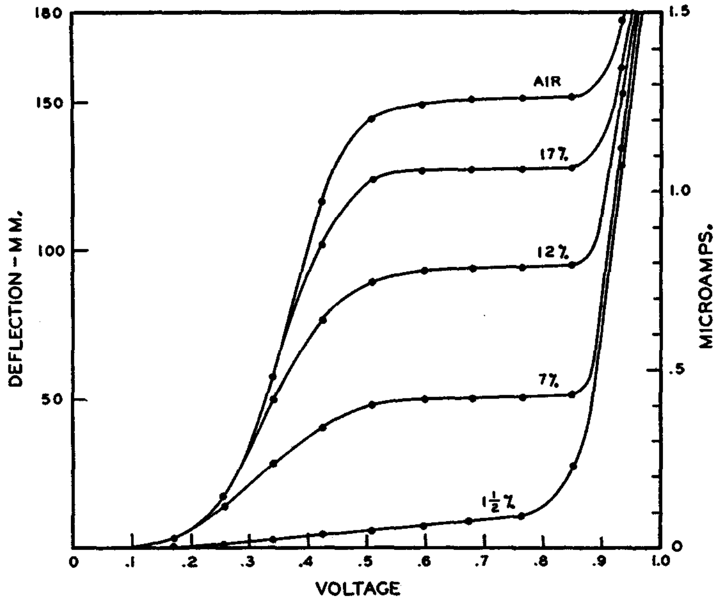

FIG. 13. Curves plotted from final point on each step of records shown in Fig. 11.

A typical set of current voltage records for M/10 KCl solutions saturated with tank N_2 (1½ per cent, O_2), 7 per cent O_2, 12 per cent O_2, 17 per cent O_2, and room air (20.9 per cent O_2) is shown in Fig. 11. Polarograms plotted from the final deflection value at each step are shown in Fig. 13. Attention is called to their similarity to ideal polarograms. The resulting calibration curve for this experiment is shown in Fig. 14 where the deflection or current at 0.7 volt for each solution is plotted against percentage saturation. The

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[4] Oxygen concentration where presented as per cent oxygen refers to saturation at atmospheric pressure.

While the method actually measures pO_2, or oxygen tension, for purposes of comparison it is frequently more convenient to give the resulting current in a μa or galvanometer deflection.

linearity of the curve appears good, but it should be called to attention that galvanometer deflection can be read to less than 0.5 mm. (or 0.3 per cent), while the known percentage saturation of the calibrating solutions depends upon analysis of the gas mixtures and is accurate in this case only to the order of 1 per cent.

Stability.—Stability experiments with air-saturated M/10 KCl in the cuvette provided a point-to-point stability within a few tenths of a millimeter with the exception of an initial and diminishing decay of about 10 per cent extending over the first minute or two interval after the circuit is closed. Long period stability experiments during several hours with the same electrode gave a day-to-day stability ±1 per cent for air-saturated M/10 KCl. Typical examples are found in Fig. 17 and in the blank in Fig. 15. Not all electrodes provided such good results, however, and the variability in electrode behavior suggests some difference in the nature of the surface and its adsorbed gas film or the bonding between metal and glass. Electrodes often functioned perfectly for a period of months and then became useless because of irreproducible variation. These were easily replaced by spares which were always kept available.

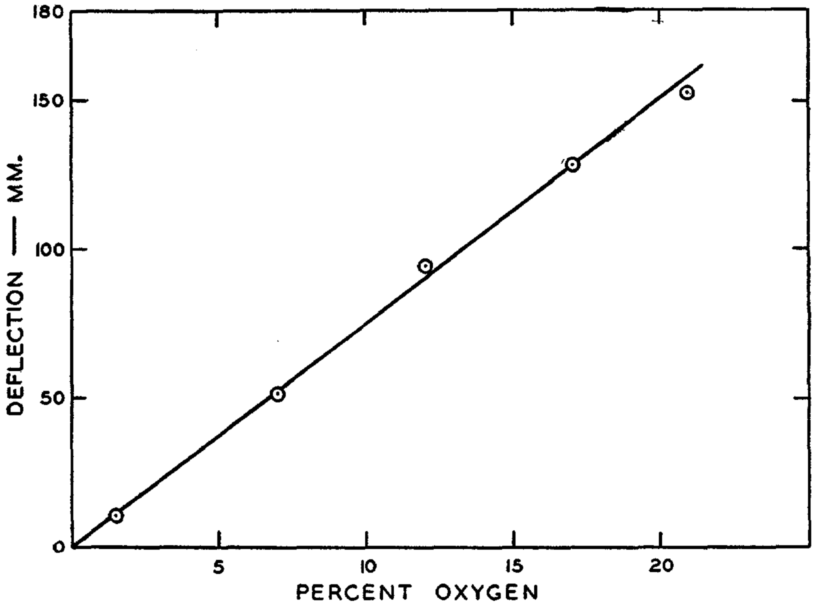

FIG. 14. Calibration curve.

Respiration Experiments with Yeast.—*Saccharomyces cerevisiae*, strain 10275 obtained from the American Type Collection, Washington, D. C., and cultured on dextrose agar at 25°C. was removed from the agar and the cells washed twice by centrifuging. After transferring to filtered M/10 KCl + 2 per cent glucose buffered at pH 6.4 by M/10 phosphate buffer to make a suspension of the desired concentration, the suspension was then placed in the cuvette and air-saturated by bubbling prior to closing the cuvette. During this interval the platinum electrode was kept suspended in M/10 KCl to prevent drying out and the formation of salt crystals on the surface of the platinum. After closing the cuvette the rate of oxygen uptake was then recorded by the Cambridge galvanometer as described previously. Typical resulting curves are shown in Fig. 15.

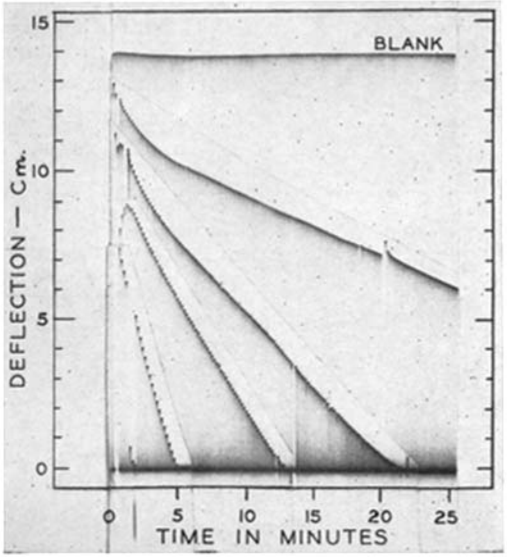

FIG. 15. Composite record of oxygen uptake by yeast suspensions in M/10 KCl buffered at 6.2 + 2 per cent glucose. Suspension of 2 × 10^8 cells per cc. diluted 1:0, 1:1, 1:2, 1:5.

Limitations of the Method.—In order to carry out valid measurements of oxygen uptake in a suspension of living cells the supporting medium or solution must provide the conditions of pH, total salt concentration, and composition within the range required to maintain normal metabolism by the material studied. The polarographic technique of analysis also makes certain requirements of the supporting medium in order to obtain conditions for diffusion-limited ion discharge. Its application to analysis of the medium surrounding living cells is therefore limited to the range where the biological and the electrochemical demands overlap. This range, of course, will vary with the requirements of the particular biological material investigated.

In the case of yeast respiration, for example, a pH range from 4.5–7.0 is desirable for uninhibited growth and respiration. There is, however, a definite lower limit to the pH which provides reliable polarographic data with platinum electrodes. In the typical polarogram with these electrodes current increases with increasing voltage until discharge is limited through ion concentration by diffusion to the electrode. The diffusion-limited current then levels off to a plateau until the potential for H^+ ion discharge is reached. The current at this point increases sharply in proportion to hydrogen discharge and masks that due to oxygen discharge. The hydrogen discharge potential is dependent upon the concentration of H ions and its intercept value at zero current is lowered with increasing concentration in the manner of a hydrogen pH electrode. The useful pH range of oxygen analysis is thereby limited to the range in which the oxygen diffusion-limited current is not masked by the hydrogen discharge current. In addition, the pH change due to CO_2 evolution, etc. during an experiment, must not be great enough to produce hydrogen discharge at the potential chosen for the measurement of oxygen concentration on the oxygen diffusion-limited current plateau. The supporting medium must therefore be buffered at a pH sufficiently alkaline to avoid the interference of hydrogen discharge current, and with sufficient buffer capacity to tolerate the change introduced by the total CO_2 production during an experiment.

TABLE I

Current due to H^+ Ion Discharge

pH	0.51 volt	0.59 volt	0.68 volt	0.76 volt	0.85 volt
8.20	0	0	0	0.1335	1.482
7.51	0	0	0.0089	0.978	+Off scale
6.72	0	0	0.0534	Off scale	" "
5.78	0	0.01781	0.623	" "	" "
4.95	0.0089	0.0623	0.712	" "	" "

Current in microamps. for representative applied potentials at different values of pH. Measurements carried out in buffered solutions oxygen-depleted by yeast suspensions.

Table I shows the results of a series of experiments carried out with yeast suspensions of about 2×10^8 cells/cc. at 16.2°C. in M/10 KCl buffered at pH 8.20, 7.51, 6.72, 5.78, 4.95 with phosphate buffer at the concentration of M/10. The suspensions were allowed to remove oxygen from the solution until the current deflection at 0.59 volt reached zero or approached a constant value. A C-V curve of each buffered, oxygen-depleted solution was then recorded. The effect of increasing hydrogen ion concentration on the shifting of the hydrogen discharge potential may result in an elevation or current contribution in the absence of oxygen.

The results indicate that under the above conditions of buffering the method can be applied to respiration studies of yeast cells down to about pH 5. Background current due to H^+ discharge can be avoided at this pH by measuring the oxygen diffusion–limited current at about 0.55 volt. Current measured at a potential lower than this approaches the shoulder or beginning of the diffusion–limited current plateau and makes for instability. In like manner other organisms with different pH tolerances, medium composition requirements, CO_2 output, etc. will impose different limitations on the application of the method.

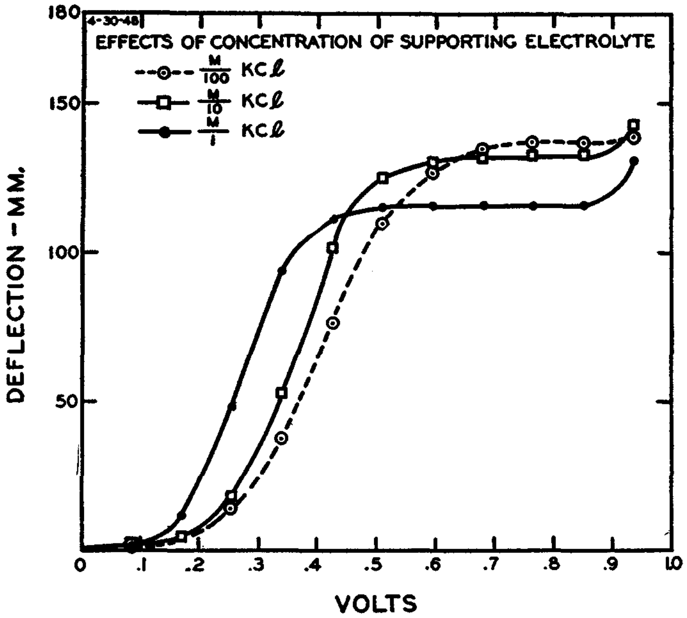

FIG. 16. See text.

The conventional polarographic technique of analysis requires a supporting electrolyte. The choice of this ion and its concentration depends upon the polarization characteristics of the ion deposited for analysis, and in the case of oxygen a solution of KCl serves effectively as a supporting electrolyte in the molar concentration range of one to one hundredth. In the case of yeast M/10 KCl is tolerated with little inhibition by osmotic effects. Such a solution was buffered at pH 6.4 by M/10 phosphate buffer and used with success in respiration experiments as shown in Fig. 15. In order to determine the feasibility of extending the application of the method to organisms with widely different osmotic requirements, polarograms were recorded for air-saturated solutions of M/1, M/10, and M/100 KCl. The results are shown in Fig. 16.

The diffusion-limited currents are in accord with predicted values for dissolved O_2 at these KCl concentrations, but the decomposition potential becomes more positive with increasing KCl. Concentrations within the entire range allow for usable polarograms, however, and the osmotic requirements of a wide variety of organisms should not limit the application of the method.

Time stability runs on blank air-saturated KCl solutions showed initial deflections decreasingly elevated above the final stable level of the curve. This initial decay prevents following oxygen changes during the first minute or two and attempts to correlate this decay with circuit and electrode characteristics were unsuccessful. Numerous variations in the ratios of pulse durations to shorting periods were explored in this respect but without significant results. The concentration of the supporting solution has a marked influence, however, as shown in Fig. 17. Low KCl concentrations minimize the decay effect while high KCl concentrations enhance it. The decay is not minimized, however, in low KCl concentrations (M/100) when yeast is present. 2 per cent glucose solution and 0.5 per cent gelatin solutions also enhanced the decay suggesting a physical limitation of diffusion restoration worthy of further study.

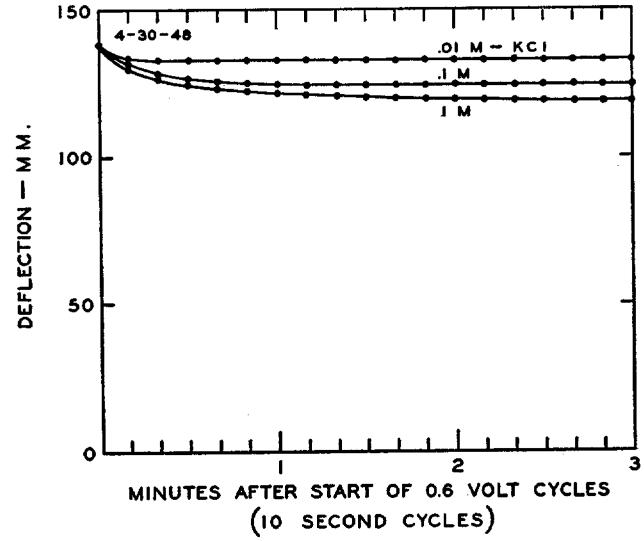

FIG. 17. See text. Effect of concentration on initial response.

The initial decay phenomenon further suggests that a similar lag may occur throughout the observations thus limiting the time resolution of the method. Results of rapidly changing the oxygen tension without disturbing the electrode or diffusion layer are shown in Fig. 18, curve *B*. This was accomplished by illuminating a suspension of green algae in the cuvette for sharply defined periods. The sharp changes between the rise in oxygen tension produced by photosynthesis during illumination and the fall produced by respiration in the dark indicate that the effect of decay on time resolution is not evident.

However, a secondary photo-effect was observed in this experiment. This is shown by the effect obtained (curve *B*, Fig. 18) when the suspension of algae, was replaced by a blank solution of air-saturated KCl. A cusp of about 10 per cent increase in total current appeared during radiation. The effect was absent in oxygen-depleted solutions and appeared to be dependent on the amount of oxygen present. It persisted when solutions containing oxygen were radiated through a "heat-transmitting" filter but was effectively eliminated by introduction of an efficient "heat-absorbing" filter such as a 15 mm. thickness of M/10 cupric sulfate solution.

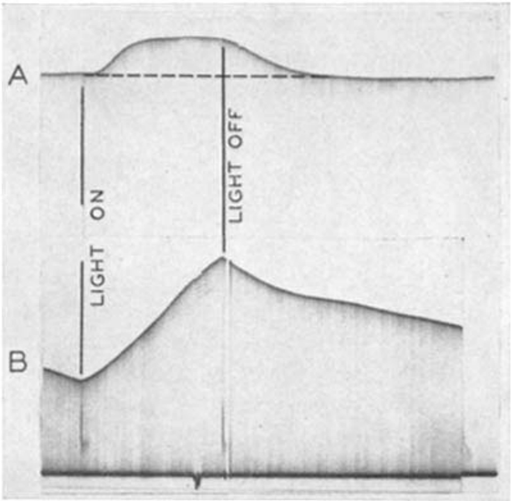

FIG. 18. Effect of visible radiation. *A*, in the absence of organisms. *B*, on suspended green algae showing time resolution and superimposed photo-action on electrode response.

Influence of Metallic Ions.—Since many metal ions such as Cu^+, Pb^+, Sn^+ etc. are deposited in the potential range of the oxygen diffusion-limited discharge in conventional polarography it becomes desirable to determine effects of these ions under the conditions of the present method. Pb^{++} was chosen for study because of the clear vertical rise at the decomposition potential and the flat plateau in the range of the oxygen diffusion-limited current of its polarogram. M/10 KCl solutions were made up with Pb^{++} at 1×10^{-3} molar concentration and diluted with M/10 KCl to 7.5×10^{-4}, 5.0×10^{-4},

2.5×10^{-4}, and 1.0×10^{-4} molar. Recorded polarograms with the apparatus for these solutions showed an almost vertical rise at 0.65 volt with an extremely flat plateau from 0.68 to 0.85 volt. Good linearity is obtained by plotting the current measured at 0.76 volt against concentration and a day-to-day stability of ±1 per cent was attained for Pb^{++} at M/1000 concentration. It is evident then that the method is sensitive to metallic ions in the same manner as oxygen and care must be taken to exclude from the supporting electrolyte those ions which are reducible in the potential range of the oxygen diffusion-limited current.

DISCUSSION

The application of a square wave potential to an electrode system is by no means a new idea, having been used for standing wave analysis of polarization and overvoltage phenomena by many investigators. Little use, however, has been made of its application to the current-time relations of diffusion-limited ionic discharge at fixed solid electrodes. In the present investigation current-time curves were studied under various conditions with the view of selecting a portion of the curve for use in oxygen analysis. The shorting period between positive and negative pulses was introduced to minimize the carry-over of current phenomena from one pulse to another. In other words the shorting period provides a "rest" interval during which charge developed by one pulse is largely dissipated before the current is developed by the following pulse of opposite polarity. This shorting period was chosen to be at least of sufficient duration to allow for dissipation of this charge to a low value. Without it current-time curves were difficult to interpret and utilize.

The exact nature of the process by which the applied potential wave form results in restoration of the oxygen diffusion gradient developed during each negative pulse is difficult to interpret from the data presented here. The time lags in reaching a stable value exhibited upon changing voltage stepwise and in closing the circuit at the beginning of an experiment both suggest that restoration is not complete with each cycle. Apparently some sort of an equilibrium is reached after a requisite number of cycles. The stability achieved in a relatively short period suggests that the diffusion layer developed is only a fraction of the thickness necessary to obtain a "steady state" in the conventionally used static platinum electrode. The present method, of course, depends upon accurate timing of commutation and measuring interval of the selected portion of each negative pulse.

The method has the advantage of utilizing simple stationary platinum electrodes with as good or improved stability as compared to those methods requiring moving electrodes, constant flow, or recessed electrodes. In addition, the mechanical and electrical system required to impose the wave form and measure the diffusion current is not costly or difficult to assemble in the laboratory.

As a microrespirometer for cell suspensions the instrument has the advantages of high sensitivity, reproducibility, and rapidity of measurement. Since it is direct reading and recording it should find application where transient changes in respiration rate of several seconds' duration are experienced. Unlike the dropping electrode it is free from the toxic effect of mercury ions. In addition its use with conventional cuvettes as respirometer vessels provides facility for simultaneous measurements of radiation or spectral absorption during respiration or photosynthesis.

It has, of course, some limitations, several of which have been discussed. The initial decay experienced during the first minute or two of a respiration experiment does not impose a hardship except in those cases where the heaviest cell suspensions are used and the O_2 uptake rate is rapid enough to have appreciably depleted the solution during this period. In all other cases it is in fact desirable to delay recording for a few minutes, in order to establish temperature equilibrium after filling the cuvette or saturating with air, etc.

During continuous oxygen uptake measurements over long periods with large or heavy cells or aggregates which settle out of suspension due consideration must be given to means of periodic stirring since measurements cannot be obtained during actual stirring.

To avoid the photo-artifact previously described precautions must be taken to filter out the intense infrared common to incandescent lamps. In preliminary studies on the effects of ultraviolet radiation on respiration no difficulty was experienced at the physiological intensities used.

The limitations imposed by hydrogen ion concentration and salt concentration are similar to those encountered with conventional platinum electrodes, and for yeast respiration have been determined and discussed above. By determinations of both the biological and the electrochemical requirements the useful ranges for each organism can be determined without too much difficulty.

As a further application, the method should lend itself to *in situ* determinations of dissolved oxygen in tissues and body fluids. In addition the absence of physical stirring should provide ideal conditions for the study of the permeability and diffusion of oxygen through membranes and tissues.

The method furthermore lends itself to scaling down to micro-electrode dimensions for micrurgical application. With present electrodes of 25μ diameter and 4 mm. length (area of about 0.32 mm.) current deflection at air saturation equals about 1 μa. This is suitable for a fast acting galvanometer but with micro-electrodes an amplifier will be required such as has been used in our oscillograph galvanometer current-time studies. With scaling down of electrodes increasing amplification is required because of diminishing current.

Correspondingly increased input resistance makes this readily possible. Since polar capacitance decreases with reduction in area as the internal resistance increases correspondingly, it is possible to provide this condition and still maintain the same *RC* constant with smaller electrodes. No difficulty in this direction is evident from present experiments and further work is projected along these lines.

In addition to application in biology the method should be of advantage in polarographic procedure in which mercury becomes undesirable such as unattended control measurements in industry or in the laboratory where anodic oxidation is to be studied.

SUMMARY

1. The possibility of obtaining sustained and reproducible results in the analysis of dissolved oxygen with simple platinum electrodes by means of the application of a periodic potential pattern was explored over a wide range of frequencies and with a variety of wave forms.

2. Satisfactory results were obtained by the application in the frequency range of 5 to 10 C.P.M. of a square wave consisting of a positive and a negative pulse with interposed shorting periods and observing the current flowing at the end of each successive negative pulse. This was found to be linearly proportional to O_2 concentration for a pulse duration of the order of 1 second when the *RC* constant of the circuit was sufficiently small.

3. An instrument was developed to provide the required wave form and record the terminal currents of the negative pulses. The instrument provides either for recording of current voltage curves (polarograms) or for continuous recording at a fixed voltage of diffusion limited current values.

4. Typical measurements of oxygen uptake with yeast suspensions illustrate the application of the technique to problems requiring frequent determinations during short intervals.

5. Applications of this technique to biological and other problems are indicated with its limitations.

Addendum.—In using solutions in the cell which differ markedly in molar concentration from the M/10 KCl solution in the salt bridge arm, it is desirable to introduce an agar plug made up with KCl. It is also desirable to eliminate pressure effects which may disturb the plug and force the bridge solution by the plug and into the cell.

In a recent application the bridge is connected and introduced by means of an agar plug at the bottom of a small tube with the electrode closing the top. This allows for a cell only 2 mm. in diameter and about 6 mm. long.

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